METHODS IN ROOM TEMPERATURE LUMINESCENCE ANALYSIS

BY

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METHODS IN ROOM TEMPERATURE LUMINESCENCE ANALYSIS

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The applications and fundamental aspects of room temperature luminescence analysis are discussed. In this investigation, a general method has been developed for solid substrate room temperature phosphorescence analysis for qualitative and quantitative determination of a variety of polyaromatic hydrocarbons, para-aminobenzoic acid, and carbazole. Whatman No. 1 filter paper was modified with thallium lauryl sulfate. The optimum heavy atom concentration of thallium(I) was determined and trace level detection of analytes was accomplished. Analytical figures of merit have been presented and limits of detection were compared to values reported in the literature determined by the same analytical technique with a variety of experimental conditions. Effects of the background emission of the solid substrate, i.e., stability and reproducibility, were addressed, and optimization of the experimental parameters has been presented.

Solid substrate room temperature phosphorescence analysis of

anthracene, a well-known weak phosphor, was successfully accomplished and reported for the first time by use of the newly developed method. Thallium(I) in the form of thallium lauryl sulfate and thallium nitrate were used to pretreat Whatman No. 1 filter paper, and the two microenvironments were compared with respect to their effect on the analytical determination of this compound. Analyte signal and background emission degradation curves for the two environments are presented. A comparison of analytical figures of merit is presented.

High background radiation is a serious problem for luminescence analyses in that it can adversely affect the reproducibility and sensitivity of the analytical determination. The analyte signal of 1,3-dimethylxanthine was moved away from the matrix background emission by an energy transfer process. Excitation energy was transferred from 1,3-dimethylxanthine to europium (III) which luminesced in a region far removed from background emission of the sample matrix. The enhanced luminescence was utilized for the quantitative analysis of 1,3-dimethylxanthine. The methodology for the energy transfer process and the analytical figures of merit for 1,3-dimethylxanthine are presented. The selectivity of this process was supported by the selective determination of 1,3-dimethylxanthine in a mixture containing 1,3,7-trimethylxanthine, a well-known interferent.

CHAPTER 1

ASPECTS OF ROOM TEMPERATURE LUMINESCENCE

Introduction

Although luminescence was observed from molecules as long ago as the 1500s, it was not until 1852 that the mechanisms for the absorption and emission process were described [1]. Today luminescence spectroscopy is recognized as one of the oldest and most established analytical techniques. Although there are several branches of luminescence spectroscopy, i.e., bioluminescence, chemiluminescence, electroluminescence, fluorescence, and phosphorescence, it is the latter two that have been most developed for analytical use [2-6]. Recent developments in luminescence methods at room temperatures, especially phosphorescence, have helped to make these particular techniques more available to researchers and analysts.

The inherent sensitivity and selectivity of room temperature phosphorescence and fluorescence analysis has rendered them applicable to the trace analysis of numerous compounds of biological and environmental interest. In the event of a choice between different analytical methods for quantitative analysis, luminescence spectroscopy has become a viable option. Polynuclear aromatics, drugs, and pesticides have been analyzed by these techniques. Several review articles have been published which include these as well as other applications [5-10]. The excellent sensitivity of these methods is exemplified by limits of detection which

range between a few thousands and one tenth part per million. Additionally, many compounds exhibit large linear concentration ranges as compared to other spectrophotometric methods, such as absorption spectrometry [11]. The selectivity of these techniques emanates from the fact that not all compounds will emit radiation and for the compounds that do luminesce, each possesses its own characteristic absorption and emission spectrum as well as a characteristic lifetime. Thus, there are three ways in which to discriminate among compounds. Even though the advantages of using room temperature phosphorescence analysis are impressive, these techniques, especially phosphorescence, have only limited acceptance by researchers and analysts for routine analysis.

Although fluorescence spectroscopy has been used at room temperature for many years and has been important for analytical investigations in biological and medicinal chemistry, the same cannot be said for phosphorescence spectroscopy. The technology in this area has been slow due to the fact that the phosphorescence process does not normally occur in solution at room temperature because of intermolecular collisional quenching, as well as oxygen quenching processes that lead to deactivation of the triplet state [12-13]. For these reasons, room temperature phosphorimetry has been done mostly on solid substrates. Solid substrate phosphorimetry has the advantage of greater selectivity and small sample requirements, both of which make this type of analysis extremely important for trace analytical applications. Although a wide variety of solid substrates have been used for room temperature phosphorescence analysis, filter paper has proven to be the substrate of choice because of cost and availability. Even so, there are problems associated with filter paper.

Background emission, signal quenching due to atmospheric water, and batch-to-batch variation among the filter papers themselves can cause problems for routine analysis [13-19]. These problems are partly responsible for the numerous variations in experimental procedures reported in the literature.

A survey of the literature produces a long list of solid substrates and a variety of experimental conditions that can be used for room temperature phosphorescence analysis for numerous organic compounds. Such variety can produce confusion and difficulty in the choice of method for the experimentalist. The need for a general method for room temperature phosphorescence analysis would make the method more amenable for routine use.

A common problem for many luminescence measurements is the background emission of the sample matrix. This problem exists for analyses done in solution or on solid substrates and can affect the precision, accuracy and sensitivity of the measurement. One way to eliminate background interference is by energy transfer from one molecule to another entity which in turn luminesces in a region removed from the interferences [3,20-22].

In this investigation, a general method to universalize the experimental conditions for RTP analysis will be developed and examined. This study will specifically address the problem of reproducibility of RTP on a solid substrate. In addition, a problem of severe background emission from the sample matrix and spectral interference between two structurally similar compounds will be addressed by a study in solution

utilizing energy transfer. Here, sensitivity, as well as selectivity, will be addressed.

Theory of Luminescence Spectroscopy

Excited State Processes

Luminescence is radiation of 200-700 nm that has been emitted by a molecule that has returned to the ground state after absorption [1-3]. This luminescence is characterized by the nature of the absorbing radiation and its relation to the ground state of the molecule. If the absorbing radiation comes from an external source, the resulting radiation is termed luminescence. Two specific luminescence processes which are currently used by analytical chemists for qualitative and quantitative analysis are fluorescence and phosphorescence spectroscopy. These processes are best described by the Jablonski diagram as illustrated in Fig. 1.

Most organic molecules have an even number of valence electrons that are paired in various orbitals. The pairing of the electrons with respect to opposing spins is referred to as a singlet state. Each state has several vibrational levels that can be occupied under various conditions. The lowest vibrational level (V_o) of the singlet state (S_o) is commonly referred to as the ground singlet state and is the most likely to be populated at room temperatures under normal conditions. Absorption of the appropriate photon of energy by a particular molecule converts the ground singlet state to an excited singlet state (S_n). Because the molecule can absorb energy corresponding to any or all of the vibrational levels of S_n, the absorption bands are usually observed to be broad. This is a fast process, on the order of 10^{-15} s. The initial excited state (S_n) that is

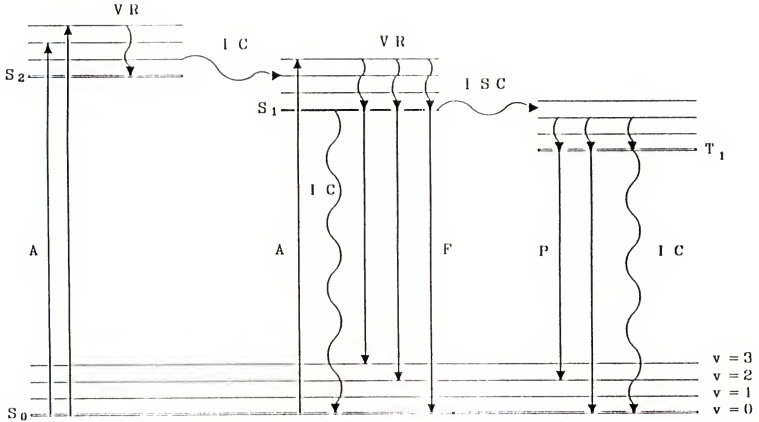


Figure 1. Jablonski diagram depicting the radiative and nonradiative processes that can occur in a molecule after absorption of radiation. (A, absorption; S_o, ground state; S_1 and S_2, electronically excited states; T_1, lowest excited triplet state; ISC, intersystem crossing; IC, internal conversion; VR, vibrational relaxation; V, vibration levels; F, fluorescence; P, phosphorescence)

formed after absorption generally undergoes rapid vibrational relaxation, 10^{-13} s or less, to the lowest vibrational level of the excited state, the extra vibrational energy being absorbed by other bonds in the molecule. More energy is rapidly given up to other vibrational modes in the molecule or by collisions with solvent molecules for internal conversion from S_n, V_o to a vibrational level of S_1. Subsequently, this excited vibrational state gives up vibrational energy until it resides in the lowest vibrational level of S_1. The change from S_n, V_n to S_1, V_o is of the time scale of approximately 10^{-11} s. Residence time of the molecule in the lowest vibrational level of the first excited singlet (S_1) state is about 10^{-8} to 10^{-7} s. Because the lifetime of this excited state is relatively long as compared to the previous processes discussed, several possible events can occur.

First, the simultaneous emission of a photon and electronic transition from S_1, V_o to S_o, the ground state, can result in the process referred to as fluorescence. It is imperative that the vibrational levels of S_o and S_1 do not overlap and that an appreciable energy gap exist in order for this process to occur. If the photon emitted is of the same energy as that which was absorbed initially, the process is termed resonance fluorescence. Generally, the fluorescence emission occurs at lower energy, i.e., longer wavelength, than the initial absorption, because of the loss of energy in vibrational relaxation and internal conversion processes before emission occurs. The time for fluorescence to occur is on the order of 10^{-6} to 10^{-8} s. In the event of overlap of the ground and excited state vibrational levels, nonradiative relaxation of the excited state to the ground state will occur.

A process that may compete with fluorescence is that of intersystem crossing (ICS). ISC necessitates a change in spin multiplicity, from S_1, V_o to the excited triplet state (T_n) and occurs on a time scale of 10^{-8} to 10^{-7} s, which is similar to the time scale for fluorescence. In order for ISC to occur, overlap of the vibrational manifolds of the S_1, V_o and T_n excited states is required. In addition, T_1, V_o must lie lower in energy than the S_1, V_o state due to the energy required in spin pairing two electrons. After vibrational relaxation to the T_1, V_o state, the excited molecule can return to the ground state by radiationless deactivation or by phosphoresence emission, or undergo reverse ISC to S_1, V_o where it can return to the ground state by either radiationless deactivation or delayed fluorescence. Phosphorescence requires a spin-forbidden transition and thus has a longer lifetime than fluorescence [3]. The term "spin forbidden" is derived from the quantum selection rules which dictates that transitions involving a change in the spin multiplicity are not allowed. The forbidden nature of spin changes and the low probability of their occurrence leads to the longer lifetimes observed for phosphorescence relative to fluorescence. The time required for phosphorescence emission is on the order of 10^{-4} s to several minutes, while that for fluorescence emission is 10^{-8} to 10^{-6} s. The average lifetime of the radiation is defined as the time taken for the intensity to decrease by 1/e.

Other Processes that Compete with Luminescence

In order for a process to compete with a normal radiative process, such as phosphorescence or fluorescence, it must be able to occur within the lifetime of the luminescence emission [3]. The lifetimes of the initial excited singlet state and the lowest excited singlet and triplet

states are such that allow for other processes to compete with luminescence. The initial excited singlet state is more limited than the other two excited states in that its lifetime is very short, approximately 10^{-13} s. Few reactions can occur within this timeframe. Therefore, the lowest excited singlet and triplets states are considered to be the most reactive sites for processes to compete with luminescence. Other than intersystem crossing and internal conversion, there are two other important events that can compete with luminescence, chemical reactions and energy transfer.

Photochemical reactions are possible by collision involving molecules in S_1, V_o or T_1, V_o excited states and other molecules. Such reactions can be observed between the excited molecules and solvent molecules or impurities in the sample matrix. Also, cleavage of bonds in molecules can occur in the presence of ultraviolet light to produce free radicals which can react with other molecules to produce new species. Photodecomposition of the analyte is also possible.

Radiationless energy transfer of excitation energy to an appropriate acceptor can also occur within the lifetimes of phosphorescence and fluorescence. Energy transfer can occur via intermolecular or intramolecular paths and can be either radiative or nonradiative. There are two mechanisms for energy transfer reactions, the resonance excitation or dipole mechanism. These mechanisms are discussed in detail in Chapter 4 of this dissertation.

Qualitative and Quantitative Relationships

Quantum Yield

Quantum yield or efficiency is a term used to describe a fundamental physical characteristic of every molecule that emits radiation. The quantum efficiency is intrinsic for each molecule and is dependent on the experimental conditions. Simply defined, it is the ratio of the number of photons emitted per second to the number of photons absorbed per second [1-3]. Quantum yields vary from 1 to 0; the greater the efficiency of the luminescence, the closer the value is to 1. A nonluminescent compound would have a quantum yield of 0.

Since luminescence rarely accounts for all the energy absorbed by a molecule, the processes that compete with luminescence must be considered. Nonradiative deactivation processes that occur from both the excited singlet and excited triplet states compete with the radiative process. Their effect on the quantum yield of a particular compound is best described by the relative rate constants (k_i) of the luminescence and the various deactivation processes. Equations 1 and 2 represent the quantum yields for fluorescence and phosphorescence, respectively [3].

$$(1) \quad \phi_f = \frac{k_f}{k_f + \sum_i k_i + k_{ISC}}$$

$$(2) \quad \phi_p = \frac{k_p}{k_p + k_{IC}} \quad x \quad \frac{k_{ISC}}{k_f + k_{IC} + k_{ISC}}$$

It should be mentioned that the quantum yield of fluorescence depends principally on the rates of ISC and IC, while the quantum efficiency of phosphorescence depends on competition between IC and phosphorescence emission as well as on the rate of ISC relative to fluorescence and IC

from the excited singlet state. Although the rate constants for both the radiative and nonradiative processes depend on the molecular structure of the molecules, the role of the microenvironment of the lumiphor, i.e. gaseous state, solution, or solid substrate, and subsequent perturbations, i.e. heavy atom presence or other entities, can drastically affect the radiative processes.

Lifetime of the Lumiphor

The lifetime of a lumiphor is an inherent property which can provide additional qualitative information about a particular molecule. It can be useful in distinguishing between molecules exhibiting similar excitation and emission wavelengths and is the basis for distinguishing between radiative emission of singlet and triplet states, fluorescence and phosphorescence, respectively. In general, lifetimes of lumiphores are reported as mean lifetimes. The mean lifetime of a particular lumiphor is defined as the probability of finding a specific excited molecule in the excited state after time t. That probability is given by $e^{-t/\tau}$. Equation 3 relates the luminescence intensity and the observed lifetime of a lumiphor [3].

(3) $I = I_o e^{-t/\tau}$

where

I = luminescence intensity at time t;

I_o = maximum luminescence intensity during excitation;

t = time after excitation source is turned off or removed; and

τ = the average lifetimes of the excited state.

A distinction should be made between the terminology "observed lifetime" and "natural lifetime." Observed lifetimes are those measured under a given set of conditions. Solvents, temperature, contaminants, heavy atom enhancers, and physical state of sample matrix can all affect the lifetime of a particular lumiphor. Therefore, it is imperative that the conditions of observed lifetimes be stated. The natural lifetime τ_o is experimentally observed when deactivation of the excited state occurs by phosphorescence emission only, and radiationless deactivation processes that compete with this emission are negligible. The relationship between natural and observed lifetime is given in equation 4.

(4) $\tau = \phi \tau_o$

where

τ = observed lifetime;

ϕ = quantum efficiency; and

τ_o = natural lifetime.

Relationship Between Luminescence Intensity and Concentration

Although the broad featureless bands that result from molecular luminescence emission under normal conditions of analysis are of limited use for qualitative analysis, by far the most important applications of phosphorescence and fluorescence measurements in analytical chemistry are in quantitative applications.

The most important factors linking luminescence intensity and concentration are the quantum efficiency of the lumiphor, the molar absorptivity, and the intensity of the incident radiation. Such factors are similar to those in absorption spectroscopy; therefore, the

relationships in luminescence spectroscopy can be related to Beer's Law for quantitative analysis.

The intensity of luminescence can be written as the product of the absorbed radiation and the quantum efficiency as in equation 5 [23].

(5) $I_L = k \phi_L I_A$

where

I_L = intensity of luminescence;

k = proportionality constant for efficiency and geometry of the detector system;

ϕ_L = quantum efficiency; and

I_A = intensity absorbed.

Since radiation absorbed is the difference between the incident radiation and radiation transmitted, a substitution is made in equation 6.

(6) $I_L = k\phi_L(I_o - I)$

where

I_o = incident intensity; and

I = intensity transmitted.

Dividing both sides of equation 6 by the incident intensity, I_o and reducing it to the simplest form produces equation 7 with the term I/I_o which is equivalent to the fraction of intensity transmitted in Beer's law.

(7) $I_L = k\phi_L I_o(1 - I/I_o)$

The Beer's law relationships are stated in the following equations.

(8) $A = abC = -\log T$

where

a = absorptivity;

b = path length of cell; and

C = concentration of absorber.

(9) $T = I/I_o$

Rewriting T in natural logarithm form and substitution in equation 8 yields equation 10.

(10) $I_L = k\phi_L I_o(1 - e^{-2.303abC})$

Expansion of the exponential term indicates that at low concentration, i.e., very dilute solutions all but the first term in the series expansion can be dropped. Changing the exponential term back to common logarithm, equation 11, yields a useable expression relating fluorescence intensity and concentration. Therefore, for very dilute concentrations of the absorber and narrow band irradiation, concentration will be directly proportional to the intensity of the absorber.

(11) $I_L = k\phi_L I_o(2.303abC)$

It must be made perfectly clear that equation 11 is only valid for optically homogeneous, transparent samples, such as very dilute solutions. In solid substrate luminescence analysis the substrate or matrix for the analyte is not homogeneous and usually not transparent. These facts cause complications that do not allow for the same simple expressions for the relationship between intensity and concentration to be used for quantitation. The substrate matrices generally utilized are solid materials such as silica gel, cellulose, or alumina and are high light-scattering materials. Both excitation and emission radiation are scattered extensively. In addition, excitation radiation can be absorbed

by the solid matrix as well as the analyte. Any expression that would relate intensity and concentration on solid substrates must take these facts into account. Because the photophysical processes occurring on solid substrates are complex and not yet fully understood, the theoretical expressions for quantitative chemical analysis have not been fully developed. Kubelka-Munk (K-M) theory has its roots in absorption and scattering phenomena and has been applied to densitometry and reflectance spectroscopy [5,24]. The equations derived in this theory appear to be the most pertinent to the understanding of the relationship between luminescence intensity and concentration, to date. Goldman and Goodall have done work in relating the K-M equations to absorption and fluorescence on solid substrates [25]. Zweidinger and Winefordner also have developed expressions for phosphorescence intensities in snow-like matrices, clear rigid solvents, and cracked glasses by integration of the K-M differential equations [26]. The derived expressions correctly predict the general trend for the behavior of the analytical log-log curves of intensity versus concentration without explicitly accounting for the excitation and emission light beams. The equations derived for phosphorescence intensity and analyte concentration, 12-16, result from analyte transmittance and analyte reflectance equations of K-M and appear to offer justification for quantitative analysis on solid substrates. These expressions represent the relationship of intensity to concentration on a solid, opaque substrate. Further details of the derived expressions can be found in references [25-29].

For low analyte concentrations the expression for phosphorescence intensity is given by

(12) $I_p = 2\phi_p I_o K\bar{b}\left(\frac{1}{1 + 2S\bar{b}}\right) = 2\phi I_o\left(\frac{2.3\ \epsilon\bar{b}}{1 + 2\ S\bar{b}}\right) C$

where

I_p = phosphorescence intensity;

ϕ_p = quantum yield of phosphorescence;

I_o = incident radiation;

K = fraction of radiation absorbed per average path length ($K = 2.303\ C$, cm^{-1});

$\bar{b}$ = average cell path length for diffuse reflectance, cm^{-1}; and

S = fraction of exciting radiation scattered per path length, cm^{-1} (S = function of analyte concentration).

If $Sb < 1$, then

(13) $I_p = 2\phi_p I_o K\bar{b}$

For high analyte concentrations the expression for phosphorescence intensity is given by

(14) $I_p = 2\phi_p I_o \left(\frac{\sqrt{K}}{\sqrt{K + 2S} + \sqrt{K}}\right)$

If $S >> K$ and analyte concentration is intermediate, then

(15) $I_p = \phi_p I_o$

If $K >> S$, and high analyte concentration, then

(16) $I_p = 2\phi_p I_o \left(\frac{K}{2s}\right)^{\frac{1}{2}} = 2\ \phi_p I_o \left(\frac{2.3\epsilon C}{2S}\right)^{\frac{1}{2}}$

For analytical curves of log intensity versus log concentration, a slope of unity is predicted at low concentrations, 0.5 at intermediate

concentrations, and zero at high concentrations of analyte. Quantitative analysis is thus possible at low analyte concentrations. In general, the upper concentration for the linear range is 10^{-4} to 10^{-3} M [12].

CHAPTER 2

EXPERIMENTAL CONSIDERATIONS FOR FLUORESCENCE AND PHOSPHORESCENCE SPECTROSCOPY AT ROOM TEMPERATURES

Physical Factors Affecting Luminescence

The structure of a molecule and its environment play important roles in whether or not a particular molecule will emit luminescent radiation [1,4,12,29]. Luminescence is favored by several factors such as high molar absorptivity coefficients, high energy gaps between the lowest excited singlet or triplet state and the ground state, and overlap of the vibrational levels between S_1 and T_n excited states so that ISC is more probable. These factors are related to the molecular structure of a molecule. In general, organic molecules which possess rigid, planar multicyclic structures have a high probability of radiative emission after absorption of ultraviolet or visible (uv-vis) radiation. Aromatic hydrocarbons are likely fluorescent candidates. Aromatic molecules containing heteroatoms generally emit phosphorescent radiation rather than fluorescence. Although absorption of energy is a prerequisite for luminescence, not all molecules that absorb radiation will emit radiation. Many molecules absorb radiation, but few emit luminescence. Thus, luminescence spectroscopy is a selective technique compared to absorption spectroscopy.

In order for a molecule to emit luminescence, it must first absorb radiation. The greater the amount of radiation absorbed, the greater the

amount of luminescence and the greater the intensity of the signal. For fluorescent molecules, the spin-allowed transition for electronic absorption has high probability; therefore the molar absorptivities of such molecules are generally high. The energy gap between the lowest vibrational level of the lowest excited singlet state and the ground state must be large enough so that nonradiative relaxation to the ground state is precluded. Also, the energy should be reasonably low so that the probability of photodissociation is small. Overlap of the vibrational levels between the excited singlet and triplet states favors ISC which increases the probability of phosphorescence emission.

The molecular environment of the analyte is of serious concern for radiative emission. The sensitivity of radiative emission, as well as selectivity between phosphorescence and fluorescence luminescence, can be controlled by proper choice of the environment of many molecules. Choice of solvent, variation of pH, and change in temperature can have drastic effects on luminescence. Manipulation of these parameters is frequently employed for selectivity and sensitivity in luminescence analysis. The presence of metal ions, heavy atoms, or impurities or other solutes can be utilized to suppress or enhance luminescence emission. Frequently, impurities can interfere with the analytical determinations. The physical state of the sample matrix is also of importance. As mentioned earlier, phosphorescence is not normally observed in solution. Therefore, a solid sample matrix is generally required to observe this type of luminescence. Addition of heavy atoms to the environment increases the probability of phosphorescence emission. Chelation of metal ions can also favor this type of analysis. In general, the presence of heavy atoms and chelation

of metal ions, serves to quench the fluorescence process and enhance phosphorescence emission [1].

Structure of the Luminescent Molecule

Aromatic hydrocarbons possess the most favorable structure for native radiative emission because of the delocalized π electron system of the conjugated ring [4,12,30]. In general, the lowest energy transitions in these types of molecules are produced by excitation of electrons from π-π^* orbitals. The π electrons move freely throughout the delocalized molecular orbitals and thus are less tightly held in bonding, compared to sigma electrons. Consequently, production of the excited state requires low absorption energies that usually fall within the uv-vis range of the electromagnetic spectrum. Also, because of the extended conjugation, aromatic hydrocarbons usually exhibit high molar absorptivities compared to nonaromatic molecules. These conditions favor radiative deexcitation for aromatic hydrocarbons. Conjugation in the ring structures of aromatic hydrocarbons provides structural rigidity and planarity to the molecule. Delocalization of the π electrons and high absorption of incident radiation, in general, produces intense fluorescence. Enhanced fluorescence intensities are generally observed by electron-donating ring substituents which can delocalize π electrons and increase the probability of radiative transitions.

Heterocyclic compounds and molecules that contain carbonyl groups possess nonbonding electrons and therefore have n-π^* transitions which are generally lower in energy than π-π^* transitions; correspondingly the molar absorptivities are much smaller. The result is that this class of molecules produces weaker fluorescence than aromatic hydrocarbons, but is

capable of producing strong phosphorescence due to efficient ISC to the excited triplet state. Electron-withdrawing substituents on this class of compounds as well as on polyaromatic hydrocarbons tend to diminish fluorescence and enhance phosphorescence.

In general, aliphatic molecules do not exhibit luminescence. The high energy required for σ-σ^* transitions can cause photodecomposition reactions. Sometimes members of this class of compounds can be derivatized to produce molecules that will luminesce [4,29].

Sample Matrix

Although luminescence has been observed in the gaseous, liquid, and solid states, analytical applications are usually performed in solution or by deposition and subsequent drying onto a solid substrate [3,12,24]. Analyses have also been done on powders and tablets containing various lumiphors. In general, only fluorescence emission is observed in solution. The long lifetime of the triplet state of phosphorescence allows for competitive reactions to occur. Intramolecular internal conversion processes can result in nonradiative relaxation, and intermolecular collisions may cause intensity quenching of the phosphorescence signal. For these reasons, phosphorescence analysis is generally performed on solid substrates or in the solid state, while the convenience of solution media is frequently utilized for fluorescence measurements. Fluorescence analysis has also been utilized on solid substrates.

Temperature, pH, and choice of solvent can affect the analytical results of luminescence analyses in solutions [1,29]. At elevated temperatures, i.e. above room temperature, there is an increase in the

number of molecular collisions between solvent and analyte molecules. Such interactions increase the probability of IC nonradiative deexcitation of the excited analyte molecule. Therefore, luminescence analyses are usually performed at room or lower temperatures. In general, fluorescence intensity decreases with increasing temperature. Additionally, choice of solvent and pH can affect luminescence intensity and the spectral characteristics of the lumiphor.

The choice of solvent and pH are of significance in the luminescence analysis of aromatic compounds containing acidic or basic fundamental groups. A change in the molecular structure of the analyte occurs with a change in the pH of the solvent as the weak acid or base dissociates [1,31]. The absorption and emission wavelengths for these compounds are likely to be different for the ionized and nonionized forms of the analyte. This change in structure of the analyte affects the quantum efficiencies of these types of compounds.

The effects of solvent polarity on the luminescence characteristics of a molecule are most significant for aromatic compounds containing heteroatoms. These molecules possess $n\text{-}\pi^*$ transitions that are normally lower in energy than $\pi\text{-}\pi^*$ transitions. Increased solvation of the nonbonding electrons occurs with increasing solvent polarity. The solvation effect can increase the energy of the nonbonding orbital so that shifts in the absorption and emission wavelengths to higher energy are observed. For some analyte molecules, the increase in energy for the $n\text{-}\pi^*$ transition becomes greater than for the $\pi\text{-}\pi^*$ transition. Thus, the $\pi\text{-}\pi^*$ transition probability becomes more favored. Since the molar absorptivity of this transition, $\pi\text{-}\pi^*$, is normally high, the result is increased

luminescence. In general, as solvent polarity increases, the energy for $n\text{-}\pi^*$ transitions increases and for $\pi\text{-}\pi^*$ transitions decreases.

Various solid matrices have been utilized for luminescence analysis. The earliest work in this area was done in frozen solutions which contained the analyte [2,32]. The frozen matrix thus restricted the molecular motion of the analyte, thereby reducing radiationless deactivation by IC and collisional quenching, and promoting luminescence. The difficulties of the experimental procedures and the poor analytical results due to cryogenic matrices prompted the development of room temperature luminescence techniques on solid substrates. In the recent years, a variety of solid supports have been utilized as substrates for luminescent measurements.

Silica gel, fiberglass, asbestos, sodium acetate, alumina, and cellulose are just a few of the solid supports that support luminescence [12,33-41]. Cellulose in the form of filter paper has become the solid support of choice because of low cost, availability, and success for promotion of luminescence from a large number of compounds. Although several theoretical and practical studies have been published on luminescence and solid substrates, an understanding of the physical interactions between the lumiphor and the substrate with respect to different experimental conditions has yet to be established [2,5,12,24]. A definitive mechanism could help to remove the uncertainties involved in selection of experimental conditions for analyses of different lumiphores. To date, the conditions for luminescence analysis are chosen based on observations obtained from previous analyses. Because of the numerous substrates and varied conditions employed for analysis, the analyst is

armed with trial and error methods that can be time consuming and confusing. Several explanations for interactions between the lumiphor and the solid substrate have been proposed by different investigators. From the numerous analytical procedures developed for RTP analysis, it is realized that two factors are important for phosphorescence emission, rigidity and planarity of the molecule. Three of the most commonly proposed mechanisms describing interactions between lumiphores and solid supports will be discussed.

It has been suggested that packing a matrix of a solid support with salts or other compounds can inhibit the internal molecular motions of adsorbed analyte molecules and result in enhanced luminescence emission. This concept was initially reported by Niday and Seybold and emanated from their investigation of 2-naphthalenesulfonate adsorbed on filter paper packed with inorganic salts and sugars [42]. Observation of increased lifetime of the analyte was attributed to inhibition of molecular motion as well as increased inaccessibility of oxygen permeation into the plugged interstices of the substrate matrix. Increased rigidity of a polymer matrix by inorganic fillers had been studied earlier by Deanin and co-workers [5]. They suggested inhibitions in the internal motions of the polymer matrix itself would favor enhanced luminescence. It has been suggested that this mechanism may be operative in conjunction with hydrogen-bonding in analyses of benzo[f]quinoline and other nitrogen heterocycles done by Ramasamy and Hurtubise on polymer-salt matrices [43]. Another investigation of the matrix packing effect was reported by Bateh and Winefordner [15]. Filter paper was treated with diethylenetriaminepentaacetic acid (DPTA) in an attempt to remove trace

amounts of transition metal impurities that might have contributed to background radiation of the substrate. Although background emission was not decreased, a significant increase in analyte RTP signal intensities was observed when compared to untreated paper. The enhanced signal was attributed to interactions between DPTA and the analyte and also to plugging of the sample matrix. It was suggested that DPTA made the matrix more rigid and forced the analyte to remain on the surface rather than allow it to penetrate into the cellulose fibers where excitation and luminescence emission would be prohibited.

A matrix isolation mechanism for RTP analysis has been proposed by McAleese and Dunlap which is based on the physical characteristics of wetting and drying cellulose supports [14]. Several solvents and phosphors were utilized in their work. The magnitudes of the RTP signals of each analyte dissolved in various solvents and adsorbed onto filter paper were compared. It was suggested that wetting and subsequent swelling of the filter paper allowed analyte molecules to enter the interstices of the cellulose matrix. Drying of the support would cause the pore structure to collapse, resulting in entrapment of the analyte molecule and enhanced luminescence.

The hydrogen-bonding mechanism has been frequently utilized to explain the interactions between lumiphores and substrates [36,78,44]. Wellons et al. investigated the relationship between ionic compounds and the emission of phosphorescence [33]. Ionic interactions between the analyte and the solid support were recognized as necessary for RTP emission. The principal interaction is attributed to hydrogen bonding of the ionized or polar molecule to the surface hydroxyl groups of the solid

support. Analytes with a dipositive charge spotted from basic solution produced enhanced luminescence relative to uncharged species. It was concluded that phosphorescence intensity was related to the number of ionic sites on the analyte molecule.

Different classes of compounds and their interactions with a variety of substrates have been extensively investigated using luminescence, reflectance, and infrared data by Hurtubise and coworkers [36,38,45-50]. A large variety of nitrogen heterocyclics and aromatic amines were spotted onto silica gel chromatoplates, filter paper, and polyacylic acid-sodium chloride (PAA-NaCl) mixtures [48]. All three supports contain active sites for potential hydrogen-bond formation. The analytes were spotted from neutral, acidic and basic solutions onto each support and the relative intensities compared. In general, analytes spotted from acidic solutions onto all supports produced the greatest signal intensities, suggesting hydrogen-bond formation between lumiphors and substrates. In other work, reflectance spectroscopy was used to elucidate the forms of benzo[f]quinoline (B[f]Q) and quinoline on the same three solid supports previously discussed [49]. Infrared data suggests several hydrogen bonding interactions for B[f]Q and phenanthrene on PAA-NaCl mixtures. Hurtubise and von Wandruszka introduced sodium acetate as a potential substrate for RTP [36]. They suggested hydrogen-bonding was responsible for RTP emission from analytes adsorbed on sodium acetate supports. Their investigation of p-aminobenzoic acid (PABA) on this support led them to postulate that hydrogen bonding of PABA anions to carboxyl groups of the support was responsible for the observed RTP emission. Dalterio and Hurtubise used multiple internal reflectance spectroscopy to study

hydroxyl aromatics spotted onto filter paper [50]. Comparison of hydroxyl group stretching frequencies for filter paper and filter paper spotted with sodium chloride or sodium bromide indicated hydrogen bonding between cellulose hydroxyl groups and the salts. After addition of the analytes, shifts in the vibrational frequencies of the cellulose hydroxyl groups suggested that more than one type of hydrogen-bonding interaction was taking place. This was supported by reflectance data. It was concluded from the study that phosphors and the support that induced RTP behave as proton donors, proton acceptors, or as proton donors and acceptors simultaneously.

Quenching Effects

Quenching is a term used to refer to those processes that reduce the luminescence intensity of excited molecules [3]. Concentration, impurities, and oxygen can cause serious quenching problems in fluorescence and phosphorescence analyses.

Inner-cell and post filter absorption effects are observed during luminescence analysis of high concentrations of lumiphors [1]. At high concentrations of solute molecules, those molecules that are nearest to the surface of the sample cell can absorb most or all of the incident exciting radiation to produce the luminescent signal. Those molecules in the interior of the cell are excited to a lesser extent or not at all. The result is decreased luminescence caused by the inner-cell effect. Alternatively, luminescence from the excited molecules can be reabsorbed by other analyte molecules, termed post filter absorption, which also results in decreased signal intensity. Another quenching process that can occur with highly concentrated solutions is the aggregation of the solute

molecules to form dimers or polymers. These species luminesce at longer wavelengths than the corresponding monomer solute molecules, thus yielding decreased luminescence signals at the monomer wavelengths. Concentration quenching is normally rectified by dilution of the sample solution.

Impurity quenching is an extremely serious problem for fluorescence analyses. Quenching of analyte signals can manifest itself by collisional interactions, energy transfer, charge transfer, and the heavy atom effect. Metal ions and other solutes can be effective singlet and triplet quenchers. Quenching can occur with or without complexation between the impurity and the solute molecule. In general, most metal complexes, including those containing aromatic ligands, do not luminesce. Hydrogen bonding between solvent and analyte molecules or impurity and analyte molecules can also affect the luminescence signal unfavorably. Probably the most ubiquitous impurity that quenches luminescence is oxygen.

Oxygen quenches luminescence intensity in solution and on solid supports and must be considered for luminescence analysis. For solution analyses, deaeration of the sample with an inert gas can decrease, but not entirely eliminate, the effect of oxygen quenching. Fluorescence analysis is the least affected by oxygen quenching. On the other hand, Phosphorescence, with its relatively long lifetime of the triplet state, is rarely observed in solution because of oxygen quenching. Schulman and Walling suggested that collisional deactivation of the triplet state was inhibited by adsorption of analytes onto the surface of solid supports, and oxygen quenching was restricted by drying [19,40]. Later Schulman and Parker investigated the effects of oxygen and moisture on solid supports [16]. Their results indicated that although oxygen could quench RTP, much

greater quenching was accomplished by the presence of moisture. Further work by Winefordner led to the conclusion that moisture competed with phosphors for hydrogen bonding to the surface hydroxyl groups of filter paper [15]. The moisture disrupted hydrogen bonds between the filter paper and the phosphor, while transporting molecular oxygen into the support matrix. Thus, the phosphors were less tightly held to the surface of the filter paper, and quenching by triplet ground state oxygen was more probable. Current methods for phosphorescence analysis employ removal of the solvent from the analyte by drying and measurement in inert atmospheres.

Heavy Atom Effect

As mentioned earlier, the presence of atoms of high molecular weight, i.e., heavy atoms, in the environment of a lumiphor have an effect on the luminescence process. In general, the presence of heavy atoms tends to diminish the quantum efficiency of fluorescence and enhance ISC to the triplet state. These effects have been attributed to increased spin-orbit coupling between the heavy atom nuclei and the electronic environment of the analyte [51-54]. Although radiative emission is frequently observed, nonradiative processes have also been reported [55-57]. The "internal" heavy atom effect was first noted by McClure in 1949 with heavy atoms bonded to luminescent analyte molecules [51]. The efficiency of this process was recently studied by Donkerbroek and co-workers in their investigation of bromo-substituted naphthalenes solvents of different polarities [58]. In 1952, Kasha published the first report on the external heavy atom effect [53]. An investigation of 1-chloronaphthalene mixed with ethyl iodide resulted in increased

phosphorescence emission. Since this publication, several investigators have studied the heavy atom effect on phosphorescence and its applications in analytical chemistry. Hood and Winefordner utilized ethyl iodide in ethanol mixtures as an external heavy atom to probe the sensitivity and selectivity of low temperature phosphorimetric analyses [59]. Winefordner and coworkers also established the principal method for inducement of RTP from nonpolar compounds by use of external heavy atoms [55]. Important biological compounds responding to a heavy atom environment were also studied by several investigators [60,61,62]. Later, heavy atom perturbation was further extended to analyses of polynuclear aromatics as well as polyaromatic hydrocarbons [63-65]. Ultimately, heavy atom perturbation has become a process associated primarily with RTP analyses.

Instrumentation

Configuration and Operation

A simple diagram of a Perkin-Elmer LS-5 Luminescence Spectrofluorometer (Perkin-Elmer, Norwalk, CT) is depicted in Figure 2 [65]. The output of this device is coupled to a model CLS-3600 data station computer. This spectrometer measures the fluorescence Xor phosphorescence of luminescent samples either in a continuous scan over the selected wavelength range (250-800 nm) of interest or at preselected points within the range. The source is a pulsed xenon flash tube that is of high intensity, short duration, and covers the ultraviolet-visible region. Energy from the source is collimated and reflected onto the entrance slit of the excitation monochromator (1200 lines per mm grating). All wavelengths of energy are diffracted by the grating, and a portion is passed to the beam splitter. At the beam splitter, a small fraction of

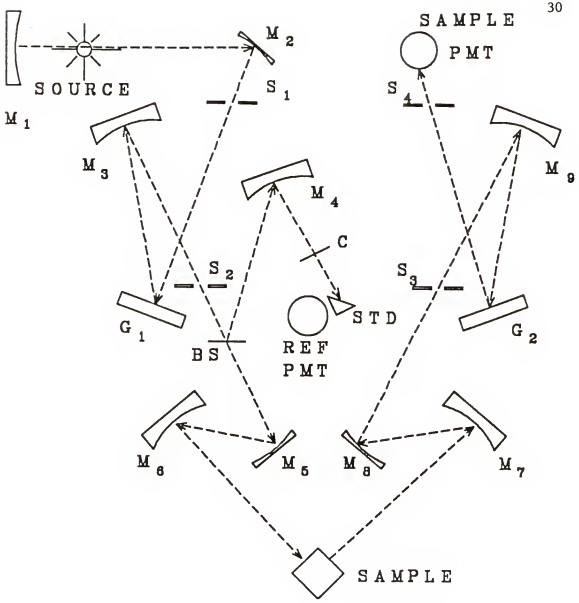


Figure 2. Instrumentation diagram of Perkin-Elmer LS-5 spectrofluorometer.
M, mirror
S, slit
G, grating
BS, beam splitter
C, comparator
REF PMT, reference photomultiplier tube
SAMPLE PMT, sample photomultiplier tube
STD, standard

the energy is directed through a compensator onto the reference cell containing a solution of Rhodamine 101 dye. The compensator compensates for the varying reflectivity of the beam splitter. The rhodamine dye absorbs energy in the 230-630 nm range and fluoresces at about 650 nm with constant quantum efficiency. This fluorescence is directed onto the reference photomultiplier detector (pmt). Simultaneously, the major portion of the excitation beam is directed onto the sample. The resulting emission from the sample is focused by the mirrors onto the entrance slit of the emission monochromator (960 lines per mm grating). A narrow band of energy is diffracted by the grating, through the exit slit and onto the sample pmt. The outputs of both the sample and reference pmts are processed by ratio-recording electronics to produce an output that is proportional to the emission from the sample that has been corrected for variations in the intensity of the source with respect to wavelength. The emission intensity and corresponding excitation and emission wavelengths selected by the monochromators are indicated digitally and the corresponding outputs can be read from a printer (digital) and recorder or computer (analogue).

Time discrimination is used to distinguish between fluorescence and phosphorescence emission. Since the lifetime of phosphorescence is long relative to fluorescence a pulse delay (t_d) is used to allow collection of the phosphorescence signal after the emission of fluorescence radiation. The events that occur in the phosphorescence mode during the excitation of a luminescent sample are shown in Figure 3 [66]. The pulse of the xenon lamp produces a band of energy with a width at half peak intensity of less than 10 μs. During this period, phosphorescence intensity

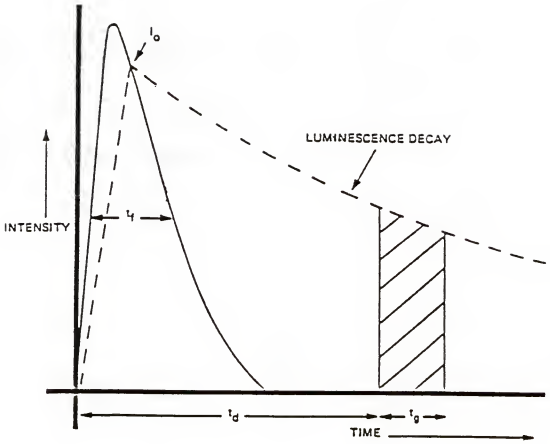


Figure 3. Schematic of events occuring during the excitation of a sample with a pulsed xenon source in the phosphorescence mode of the LS-5 spectrometer.

increases to a maximum (I_o) and subsequently decays exponentially to zero. A delay time (t_d) for the sample pmt is set so that only phosphorescence emission is collected. A sample gate time (t_g) is set for the time of data collection. If t_d is set to 1 ms, which is well within the lifetime for phosphorescence, no fluorescence emission is measured. Detection of fluorescence emission is accomplished by removal of t_d.

Analytical Applications of Fluorescence and Phosphorescence

Fluorescence analysis was the first and most widely used of these two luminescence techniques for analytical determinations. Initially this method of analysis was utilized extensively by molecular biologists in the study of amino acids, proteins, vitamins, and enzymes. Current applications of fluorescence analysis are rooted in biochemistry as well as clinical chemistry [4,5,7,67]. Applications are numerous and can be found in the previous references. Applications include the studies of drugs, toxic agents and metabolites and their effect on the body as well as inorganic substituents in blood and serum.

Although phosphorescence had been observed many years ago, analytical applications have been sparse until the last 20 years. Initial analyses were performed at low temperatures, requiring the use of cyrogenic equipment and tedious experimental procedures. The difficulty of these measurements and the problems associated with the procedures most probably deterred rapid advances in the use of phosphorescence as an analytical technique for routine analysis [2]. The development of phosphorescence analysis at room temperature coupled with the additional selectivity offered by the technique makes RTP an attractive method for trace chemical analysis [2,4]. Because the development of this technique

is relatively new, the major portion of published work in this area is mainly of theoretical nature. Many investigations have been done on pure systems and applications have been slow in development. Primarily, RTP applications have been centered on analyses of various drugs in pharmaceutical preparations [35,68]. Characterization of synfuels and coal-related materials by RTP has been reported [68,69]. In addition, polynuclear aromatics have been quantitated in air samples by RTP [69,70].

CHAPTER 3

ROOM TEMPERATURE PHOSPHORESCENCE ON SOLID SUBSTRATES

Background

Room temperature phosphorescence spectrometry is a relatively new analytical method that is utilized for the analysis of trace components in biological, pharmaceutical, and environmental samples [12]. Although RTP analyses have been performed with analytes in the solid, liquid, and gaseous states, the simplest method of analysis is done with the analyte in the solid state on a solid support. Reports on solid surface RTP appeared in the literature in 1896, but until the work of Roth, Schulman, and Walling, in the early seventies, the full potential of this technique was not recognized [19,40,41]. Roth was first to report RTP emission from several compounds analyzed on a cellulose substrate. He reported microgram limits of detection. Schulman and Walling extended this work to other solid supports, such as silica, alumina, paper, and asbestos. They were first to report on interactions between the phosphor and the solid substrate. They reported that the adsorption of the molecules on the solid substrate provided rigid conditions that inhibited collisional quenching and radiationless deactivation of the triplet state. It was noted that the ionic nature of the analytes was frequently important for the observation of RTP. Since then, numerous investigations of solid substrates RTP analyses have been reported in the literature.

Vo-Dinh and Hurtubise have recently reviewed the theoretical aspects of RTP phenomena on solid substrates in detail [12,24,71]. Recently, Schulman has published two excellent books which include current methods and applications of this technique [4,5]. In addition, excellent review articles by Wehry have been published in analytical chemistry [8,9].

Solid surface phosphorescence at room temperature involves the methodology and theory concerned with phosphorescence emission emanating from compounds adsorbed on solid matrices, as well as from solid compounds such as tablets and powders. Because the technique is relatively new, several theories to explain the interactions occuring between the solid surface and analyte have been proposed. These were discussed in detail in Chapter 2. Because the number of natural phosphors is limited, an understanding of the processes which promote phosphorescence emission are essential to the development of future methods and applications. Schulman and Parker hypothesized hydrogen bonding between hydroxyl groups on filter paper and phosphors as the principle interaction for solid substrate RTP. Their conclusions resulted from significant decrease in RTP emission from phosphors spotted on silanized paper [16]. Hurtubise and coworkers have developed a method to determine fluorescence and phosphorescence quantum yields for compounds adsorbed on a number of solid surfaces [73,74,75]. Quantum yields for fluorescence and phosphorescence were determined for the anion of the PABA adsorbed on sodium acetate. This data was coupled with phosphorescence lifetime data at different temperatures for the same system to propose a "rigidly held" mechanism for the interactions between the substrate and the phosphor. The results of the study indicated that the more tightly the phosphor was held to the substrate the greater the

quantum yield. Hurtubise and coworkers suggested hydrogen-bonding interactions between polymer salt mixtures, sodium acetate, and filter papers and nitrogen heterocycles [36,43,48]. The suggestion of a "rigidly held" phosphor promoting phosphorescence is common in today's literature [12,24,17,34,76].

Solid substrate RTP is, in general, both sensitive and selective. The sensitivity of the method is well documented in the literature by nanogram and sometimes subnanogram detection of a variety of organic compounds. Selectivity in phosphorescence measurement is not only an inherent property of the molecule, but a property that can be controlled by the physical environment of the lumiphor. Many molecules absorb radiation, but not all of these luminesce. Of the luminescing molecules, a small portion will phosphoresce. Thus, this inherent selectivity of a phosphor is much greater than that for a fluorophor or absorber. The environment of the phosphor can have a serious effect on the sensitivity, as well as the selectivity, of the analytical measurement.

A great deal of work has been done on different classes of compounds, such as drugs, PAHs, PNAs, and pesticides [12]. These studies are focused on the analytical figures of merit (AFOMs) for the investigations, all of which are directly related to the experimental conditions. Experimental conditions such as choice of solvent, drying method, drying time, pH, solid support, and heavy atom enhancer are frequently manipulated in order to achieve maximum sensitivity and selectivity. Solvent choice, drying method and time, and pH are physical parameters that must be optimized for most RTP investigations.

Although a variety of solid supports, such as silica gel, fiberglass, asbestos, sodium acetate, and cellulose have had applications in RTP methods, filter paper is most frequently utilized as the solid substrate. The use of heavy atom enhancers has probably made the biggest impact on solid substrate RTP analysis because this led to the development of methods for a whole new class of compounds, PAHs which are of environmental and biological interest [62].

RTP analysis on solid supports is characterized by simple experimental methods, relatively inexpensive instrumentation, and small sample requirements. Once the method is developed for a particular analysis, phosphorescence data are usually fast and simple to acquire. The experimental procedure consists of spotting a few microliters of analyte onto a solid support and evaporation of the solvent by heat, infrared radiation, or inert gas purge. The analytical measurement is then taken with the sample in an inert atmosphere. The instrumentation for phosphorescence measurements is inexpensive in that most luminescence or fluorescence spectrometers can be readily adapted with minor modifications for phosphorescence analysis. This makes the fluorescence spectrometer especially advantageous since two analytical techniques are possible from one instrument.

It should be clear that the important advantages of solid substrate phosphorescence analyses are sensitivity and selectivity. In addition, the small sample requirements of the technique are equally important. Biological, medical, and pharmaceutical samples are frequently small and precious, thus, limiting the choice of methods for analytical

determinations. Solid substrate RTP analytical methods are an attractive choice for these types of samples.

Although numerous publications exist in the literature on the use of solid substrate RTP analysis for the analytical determination of a large number of compounds, the technique has been primarily a tool of the research scientist for theoretical studies and acquisition of data for model compounds which are necessary for development and application of the technique to analytical determination of "real" samples. Applications to "real world" samples are sparse and slow in development. Literature reports of analyses performed by this method reveal a variety of experimental conditions for the trace level determination of a large number of analytes. The numerous variations in experimental conditions frequently render luminescence analyses confusing and sometimes difficult. Also, there are problems associated with filter paper supports, which are the solid substrates most commonly chosen for RTP analyses. The objective of this research is to develop a substrate that can be utilized for RTP analysis of a variety of compounds that would minimize some of the problems associated with filter paper. Thus, solid surface RTP could be used routinely in analytical labs for trace level determinations of a variety of compounds.

Cellulose as a Solid Support

The most common solid support used for RTP analysis is cellulose filter and chromatographic paper. There are several kinds of filter paper which are conducive for phosphorescence emission from a large number of compounds. The popularity of filter paper supports for RTP analyses is due to low cost, ready availability, ease of handling, and ease of

modification. Although filter paper is advantageous in a lot of ways, it presents several problems for the analyst. These problems are principal factors for the slow development in RTP application analyses.

Problems with filter paper are well documented in the literature [12,13,72,77]. Nonuniformity in the surface, variation in the physical and chemical composition within the same batch, and sensitivity to oxygen and moisture quenching can cause serious problems in sensitivity and reproducibility of the analytical methods. A major problem is that of background emission that occurs for all types of filter papers that induce significant phosphorescence signals. A broad band of emission is produced from 300-600 nm which has been suggested by many investigators as the limiting factor for sensitivity in solid substrate RTP measurements [6,13,44,77,78]. RTP emission of many phosphors occurs within this region and consequently, their signals must be measured on top of this high background. The presence and variation of background radiation among supports of the same batch contributes to poor precision in RTP methods.

An in-depth investigation of cellulose and cellulose filter papers has been done by Bateh and Winefordner [15,44,68,79]. They suggested that hemicellulose, or possibly trace amounts of transition metals are responsible for the background radiation produced by RTP. Complete removal of the hemicellulose by extraction with dioxane resulted in the absence of analyte phosphorescence. An investigation to remove the trace metals by chelation was unsuccessful in proving their existance, but was favorable in the observation of increased analyte signal intensities [15]. Bateh and Winefordner suggested that in general, treatments of filter paper do not significantly reduce the background emission but could

improve the absorption characteristics. Several other methods to reduce the background emission of filter paper have been attempted by several investigators [12,13,80,81]. Irradiation of filter paper, solvent extraction, and chemical and heat treatments have all been investigated as possible means of decreasing background radiation. In general, irradiation has some success in background emission reduction, but the effects on RTP analytical figures of merit for different classes of compounds requires further work.

Large variations in background emission of different batches of filter paper contribute to the reproducibility problems typically associated with RTP analyses [15]. Investigations using x-ray photoelectron spectroscopy by Andino and coworkers addressed physical characteristics of the filter paper surface [82]. An electron microscope photograph of Whatman No. 1 filter paper revealed the surface as rough and irregular consisting of numerous interstices among the cellulosic fibers. Chromatographic migration of the solvent containing the analyte allows the analyte to become distributed over a large area and into the interior of the support. This migration and penetration of phosphors into the varied bulk of the filter paper can result in loss of RTP signal and unfavorable precision for replicate measurements. It was also observed from this work that both the heavy atom cations and anions were retained equally at the surface of the support, despite the fact that they are oppositely charged and their chemical interaction with the cellulose should differ. In addition, it appeared that the heavy atom penetrated into the bulk of the support to a lesser extent than the phosphor. The idea of using a heavy atom surfactant in order to increase retention of

the heavy atom near the surface was proposed by de Lima and coworkers [83]. When silver and thallium surfactants rather than the respective nitrate salts were used for RTP analysis of carbaryl on filter paper, 10-fold enhancement of RTP emission was observed.

Surfactants and Room Temperature Phosphorescence

The use of surfactants for observation of RTP of phosphors in solution was first reported by Kalyanasundaram and coworkers [84]. Several years later, Turro and coworkers developed the experimental techniques [85]. Humphry-Baker and coworkers incorporated silver ions as counter ions into micelles for heavy atom perturbation of several PAHs [86]. Cline-Love and coworkers investigated the analytical applications of micellar solutions and RTP [87-91]. Organic phosphors were incorporated into micellar assemblies where they were protected from oxygen quenching and vibrational deactivation. An attractive aspect of this technique is the ability to increase the solubility of organic compounds in aqueous solution and also increase the proximity of heavy atom perturbers for successful measurement by RTP. Current analytical investigations by Winefordner and coworkers involve the spotting of micellar assemblies of organic compounds onto filter paper for solid substrate RTP [92,93]. Several different kinds of surfactants were utilized for the analytical determination of several PAHs and carbazole. Thallium nitrate was used as the heavy atom perturber resulting in significant enhancement of the analyte signals. In other work by these same investigators, phenothiazine derivatives were studied in the presence of different surfactants utilizing two different filter paper substrates. The effect of thallium on the analyte signals was also addressed.

Although this method addresses the problems of RTP in solutions and the latter work is concerned with RTP analysis of phosphors incorporated into micellar assemblies and their analysis on solid substrates, a major advantage of solid substrate RTP has been negated. That advantage, small sample requirements has been lost in these investigations. Also the disadvantages of this solution work should be noted. Micelle-stabilized RTP suffers from several drawbacks [72]. The dissolution of nonpolar solutes in aqueous micellar solutions is slow. Some PAHs with low polarities cannot be dissolved, limiting the applicability of this method. Long sonication times and degassing of solutions are required, processes which induce significant foaming problems. The results of foaming are serious for the preparation of the analytical standards and the applications to solid substrates and are reflected in the accuracy and precision for the methods.

The following investigation is concerned with the development of a solid substrate that can be used for RTP analysis of trace amounts of a variety of compounds. The surface of Whatman No. 1 filter paper was modified with a surfactant. Since thallium(I) has been shown to be an effective heavy atom enhancer for the RTP analysis of several compounds [92-95], the thallium analogue of sodium lauryl sulfate was prepared, and the methodology for optimum preparation of the support was established. Anthracene, a nonpolar PAH, was used as a model compound to determine optimum conditions for preparation of the modified substrate. A comparative study for the analysis of anthracene was done using both TlLS and $TlNO_3$ as modifiers of the cellulose matrix. AFOMs for the two methods were determined and advantages and disadvantages were examined.

Room Temperature Phosphorescence of Anthracence on Pretreated Cellulose Substrate

Introduction

Anthracene, a nonpolar PAH, is a compound of significant environmental concern. It is a major component of coal tar and is commonly found in environmental samples such as waste waters, sediments of lakes and rivers, and air sample extracts [96,97]. Although the compound itself is not considered to be an active carcinogen, derivatives such as 2-anthramine and 1,8,9-trihydroxyanthracence are known to be active tumor promoters which prompts concern for the analytical determination of anthracene in trace amounts [98,99].

The selectivity, sensitivity, simplicity, and small sample requirement of solid substrate RTP analysis makes this an attractive choice for the analysis of this type of compound. In the specific case of anthracene, however, the weak phosphorescence emission makes its determination at the trace level difficult [12]. To date, only one successful attempt to enhance the RTP signal of this compound on solid substrates has been reported. Dried filter papers, previously impregnated in saturated aqueous solutions of alpha, beta, and gamma-cyclodextrins were tested by Vo-Dinh and Alak [100]. The greatest signal enhancement was obtained with beta-cyclodextrin. Unfortunately, analytical figures of merit were not reported in this particular investigation and the sensitivity determined with the impregnated substrate could not be evaluated. Although trace analysis of PAHs in environmental samples can often be satisfactorily accomplished by solid substrate RTP with the use

of a heavy atom, the analytical determination of anthracene by this method, to this author's knowledge, has not been reported.

Inorganic salts of the heavy atom of choice are generally used for RTP analysis of many organic compounds. However, there are several problems associated with the use of salts as heavy atom enhancers for RTP analysis. Normally the choice solvent for dissociation of the required concentration of heavy atom is limited to water. The problems of water and filter paper were documented earlier in this dissertation. Because of the long drying times associated with the usage of water, ethanol is frequently used as a solvent for RTP analysis, however, the solubility of inorganic salts in ethanol is very poor. The problems of water and filter paper result in poor precision and decreased RTP signal intensity. The migration of water on cellulose distributes analyte molecules over a large area of the cellulose support, thus decreasing the analyte concentration in the region of the excitation beam. Also, since the heavy atom is frequently applied by syringe, salting out of the heavy atom can occur in the needle of the syringe due to capillary action of the solvent and contact of the salt with the metal needle. Both factors can result in irreproducible applications of the heavy atom onto the filter paper surface. The end result is that both precision and accuracy in analytical determinations can be affected adversely. These problems coupled with observations reported by de Lima and co-workers [83] of favorable RTP signal enhancement of carbaryl in the presence of $TlNO_3$ and NaLS on a filter paper support, a surfactant containing a heavy atom was chosen as a matrix modifier for development of a substrate for RTP analysis of anthracene.

In this investigation, quantitative and qualitative analysis of anthracene was accomplished using a heavy atom perturber by solid substrate RTP. The substrate, Whatman No. 1, was pretreated with monovalent thallium, Tl(I), in the form of thallium nitrate and thallium lauryl sulfate. Two different microenvironments for the heavy atom were utilized and compared with respect to their effect on the analytical determination of anthracene.

Results and Discussion

The excitation and emission spectra of anthracene on Whatman No. 1 filter paper pretreated with TlLS are presented in Figure 4. The RTP excitation and emission maxima occur at 252 and 677 nm, respectively. The spectra of this PAH on Whatman No. 1 pretreated with $TlNO_3$ is very similar to Figure 4. Although the spectral bandpass for the excitation monochromator was 5 nm, the excitation maximum for anthracene in the presence of TlLS was consistently observed at shorter wavelength than that for $TlNO_3$ at 257 nm. The spectral data for anthracene in the presence of $TlNO_3$ and TlLS is presented in Table 1. The background radiation intensity produced by Whatman No. 1 filter paper, irradiated with light of 252 nm, before and after treatment with a heavy atom, is shown in Figure 5. The maximum emission intensity produced by 0.25 M $TlNO_3$ treated filter paper occurs at approximately 504 nm and is about 3 times that of untreated paper. Filter paper with 0.033 M TlLS produced twice the background emission intensity as $TlNO_3$ at 504 nm. Since anthracene emission is at 677 nm, problems resulting from background radiation should be minimal.

In order to obtain the optimum heavy atom concentration for the analytical determination of anthracene, the phosphorescence signal of 3

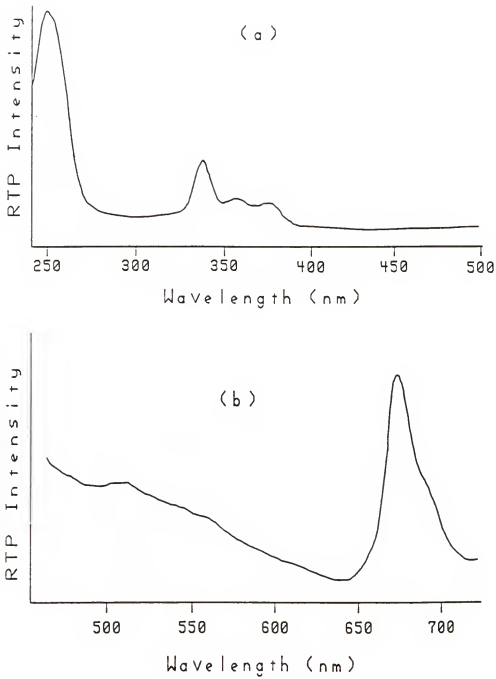


Figure 4. RTP excitation and emission spectra. (a) Excitation of anthracene on Whatman No. 1 pretreated with TlLS. Maximum excitation at 252 nm. Emission at 330 nm due to second order scatter. (b) Emission of anthracene on Whatman No. 1 filter paper pretreated with TlLS. Maximum emission at 677 nm. Spectrum uncorrected for background emission.

Table 1. Room Temperature Phosphorescence Spectral Properties of Anthracene Examined on Whatman No. 1 Filter Paper Pretreated with Thallium (I).

Heavy Atom Salt	Tl(I) (M)	λ_{ex}[a] (nm)	λ_{em} (nm)
$TlNO_3$	0.25	<u>257</u>,361,330	679
TlLS	0.033	<u>252</u>,360,378	677

[a]Wavelength for maximum RTP emission is underlined when there is more than 1 band; the precision for the wavelengths is ±0.5 nm.

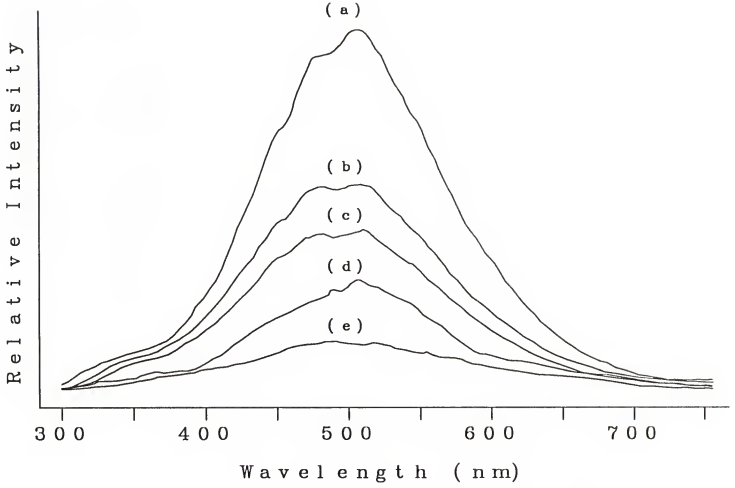


Figure 5. Background emission at excitation of 252 nm, of Whatman No. 1 filter paper pretreated with different concentrations of heavy atoms; (a) 0.13 M TlLS; (b) 0.033 M TlLS; (c) 0.016 M TlLS; (d) 0.25 M $TlNO_3$; (e) filter paper with no heavy atom.

μl of a 10 μg/ml anthracene solution was measured in the presence of different concentrations of $TlNO_3$ and TlLS. From Figure 6 it can be seen that the strongest signals occurred for concentrations of 0.033 M and above for TlLS, and for concentrations of 0.1 M and above for $TlNO_3$. All further experiments were performed using 0.25 M $TlNO_3$ and 0.033 M TlLS since these concentrations represented the optimum signal to background ratios, as well as, the best precisions for the net analyte signals as indicated in Table 2. RTP of anthracene on Whatman No. 1 filter paper without heavy atom solutions was not observed.

Two other pieces of information were gleaned from Figure 6. First, for the same analyte concentration, the emission intensity produced in the presence of TlLS was more than twice that observed for $TlNO_3$. It was also noted that the greater analyte signal intensity is produced by a smaller amount of Tl(I) when the heavy atom is in the form of a surfactant. When corrected for background radiation, 0.1 M $TlNO_3$ produced approximately the same analyte signal intensity as 0.01 M TlLS. There were two differences in the methods of analysis that may explain these observations. The solvent for $TlNO_3$ was limited to water because of solubility of the heavy atom salt, while that of TlLS was ethanol/water (80:20 by volume). Also, the anions of the thallium compounds differed significantly in their physical structure.

The difference in the solvents and the physical structure of the anions of the heavy atoms probably affected the chromatographic migration of the heavy atom solutions. The aqueous heavy atom solution spread quickly over the entire filter paper surface after deposition, in contrast to the ethanolic solution. The TlLS ethanolic solution spread in a very

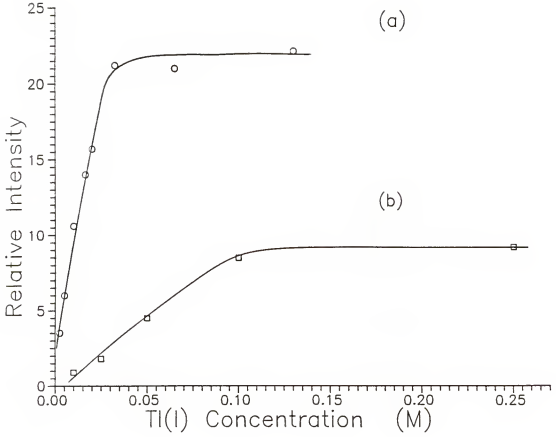


Figure 6. The effect of heavy atom concentration on RTP of anthracene; (a) Whatman No. 1 filter paper pretreated with various TlLS concentrations and 3 μl, 10 μg/ml anthracene; (b) Whatman No. 1 filter paper pretreated with various $TlNO_3$ concentrations and 3 μl, 10 μg/ml anthracene. Each point was measured in triplicate and represents net anthracene intensity.

Table 2. The Effect of Increasing Heavy Atom Concentration on Signal to Background Intensity Ratio and Reproducibility of 10 ppm Anthracene Measurement.

Heavy Atom	Concentration (M)	Signal[a] / Background[b]	Precision for Analyte[c] (% RSD)
$TlNO_3$	0.01	0.10	72.4
	0.025	0.17	65.8
	0.05	0.35	14.7
	0.10	0.70	9.06
	0.25	1.68	8.94
TlLS	0.0025	0.41	36.2
	0.005	0.52	14.2
	0.01	0.72	24.2
	0.0163	0.63	63.3
	0.02	0.97	29.8
	0.0325	1.79	8.34
	0.065	1.54	8.15
	0.13	1.41	5.7

[a]Normalized net signal intensity for microgram/milliliter of anthracene on Whatman No. 1 pretreated with heavy atom at appropriate excitation and emission wavelengths.

[b]Normalized signal intensity for RTP emission intensity of Whatman No. 1 filter paper pretreated with heavy atom.

[c]Three determinations of 10 ppm anthracene and their respective blanks were utilized to determine the precision.

small area around the point of deposition. This may be the result of combined factors. The ethanolic solution would evaporate rapidly, decreasing migration time, and the more bulky nature of TlLS would tend to migrate at a slower rate relative to the $TlNO_3$. The result would be a greater concentration of heavy atom at the point of deposition. This proposal was supported by visual observation, with the naked eye, of a crystalline network on the surface of the filter paper substrates that had been treated with high concentrations of TlLS. The physical structure of the lauryl sulfate ion is such that interactions between the neutral oxygen atoms and thallium ions is probable because of the increased size and lesser electronegativity of the sulfur atom relative to the nitrogen atom in the nitrate ion. These conditions are amenable to crystalite formation and would also contribute to a small densely populated area of heavy atoms. Crystal formation, as well as, the large anion size probably helps to hold the thallium atoms closer to the surface of the filter paper where heavy atom perturbation of the analyte and stabilization of the triplet state is possible. Also, the long alkyl chains of the anion can entrap the analyte molecule, thus restricting their molecular motions and decreasing nonradiative deactivation of the triplet state. Both processes would favor the phosphorescence enhancement that has been observed.

The phosphorescence intensity of the heavy atom and anthracene was affected by irradiation time in the sample compartment, as well as, by the environment of the thallium ion. Figures 7 and 8 represent the RTP signal as a function of irradiation time, at the maximum anthracene wavelengths and optimized monochromator slit widths, of the pretreated substrate with and without analyte in the presence of $TlNO_3$ and TlLS, respectively. Table

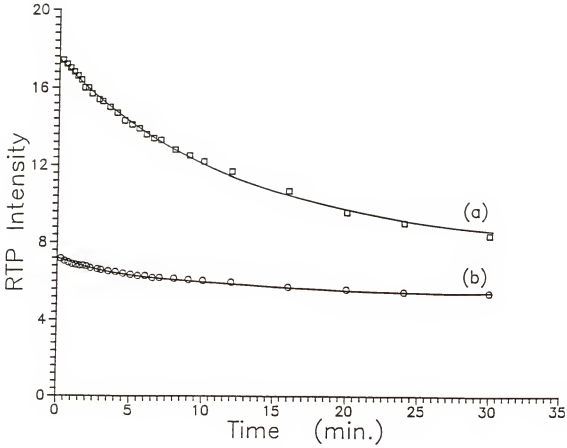


Figure 7. The change in RTP intensity with respect to irradiation time. (a) Whatman No. 1 filter paper pretreated with 0.25 M $TlNO_3$ and spotted with 3 μl, 10 μg/ml anthracene; (b) Whatman No. 1 filter paper pretreated with 0.25 M $TlNO_3$.

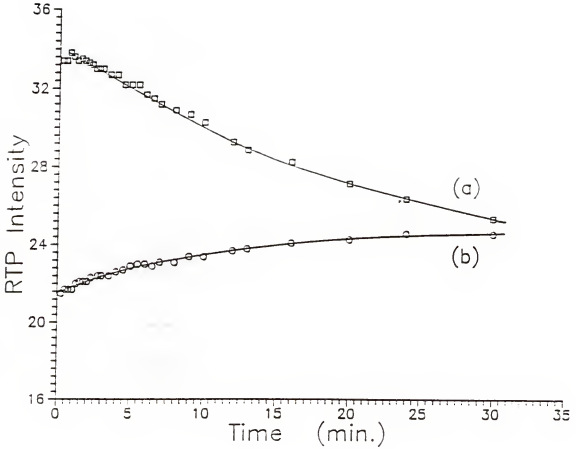


Figure 8. The change in RTP intensity with respect to irradiation time. (a) Whatman No. 1 pretreated with 0.033 M TlLS and spotted with 3 μl 10 μl/ml anthracene; (b) Whatman No. 1 filter paper pretreated with 0.033 M TlLS.

3 summarizes the change in RTP intensity of these samples with respect to irradiation time. It is observed from these data that the RTP emission intensities of analyte, as well as, the pretreated substrates undergo changes with irradiation time. The RTP signal intensity of $TlNO_3$ on Whatman No. 1 decreases approximately 10% with 5 minutes of irradiation and approximately 30% after 60 minutes of irradiation. Conversely, Whatman No. 1 pretreated with TlLS shows approximately a 5% increase in RTP intensity with 5 minutes of irradiation and approximately 14% increase after 60 minutes. Irradiation of Whatman No. 1 filter paper without either heavy atom present under identical experimental conditions produced negligible change in phosphorescence intensity. The observed decay of the $TlNO_3$ RTP intensity may be due to reduction of Tl(I) to Tl(0) by the intensity and time of irradiation. The increase in TlLS RTP intensity can be explained by increased drying caused by increased residence time in the nitrogen atmosphere and longer exposure to source irradiation. Moisture and molecular oxygen which quench RTP signals may be entrapped in the long alkyl chains of TlLS. Once these entities are removed, the alkyl environment should be more resistant to atmospheric moisture and should offer protection to the heavy atom, as well as the analyte, from adverse effects due to source irradiation.

Addition of anthracene to the pretreated substrates and subsequent irradiation by the source in the sample compartment of the spectrometer, produced significant reductions in the RTP emission intensities of the analyte. Since the reduction in the signal intensities of the substrates containing the analyte were significantly greater after 5 minutes of irradiation than for the pretreated substrate alone, the reduction in

Table 3. Percent Decrease in RTP Intensity of $TlNO_3$, TlLS, and Anthracene With Respect to Irradiation Time

Irradiation Time (min)	I_1 (%)	I_2 (%)	I_3[a] (%)	I_4 (%)
1	-3.3	-3.4	+1.0	-0.60
5	-9.9	-18.0	+5.5	-3.6
10	-14.0	-19.0	+7.8	-9.3
30	-23.0	-51.0	+13.0	-24.0
60	-28.0	-62.0	+14.0	-33.0

I_1 = Whatman No. 1 and 0.25 M $TlNO_3$

I_2 = Whatman No. 1, 0.25 M $TlNO_3$, and 10 μg/ml anthracene

I_3 = Whatman No. 1 and 0.033 M TlLS

I_4 = Whatman No. 1, 0.033 M TlLS, and 10 μg/ml anthracene

[a]Percent increase in RTP signal with respect to irradiation time.

signal intensity is most likely attributed to photochemical reactions involving anthracene. It has been reported that ultraviolet irradiation of anthracene in coal tar naphtha produces a dimeric species. [101]. This species may phosphoresce under the experimental conditions used or it may emit radiation at a wavelength different than was used for the analyses.

It is observed from Figures 7, 8 and Table 3 that the most significant reductions in anthracene emission intensity were observed by irradiation of the analyte on the substrate pretreated with $TlNO_3$. It was observed that for 5 minutes of irradiation time, the anthracene RTP intensity produced by the $TlNO_3$ substrate decreased approximately 18% as compared to a 4% decrease observed for the TlLS substrate. After 30 minutes of irradiation, the RTP intensity of anthracene on Whatman No. 1 pretreated $TlNO_3$ decreased 50% in contrast to a 24% decrease in emission intensity observed on the TlLS substrate. Since 5 minutes is the approximate time to take a spectral scan, this degradation of signal intensity is significant and can affect the precision of the measurements. The standard deviation observed for the net RTP signal for triplicate measurements of anthracene on Whatman No. 1 pretreated with $TlNO_3$ and TlLS was 17% and 7%, respectively, for 5 minutes of irradiation time. Therefore, for measurement of all analytical curves, the excitation and emission monochromators were set at the appropriate wavelengths, and the RTP intensities were read at approximately 1 minute of irradiation time. In this manner, irradiation of the sample and subsequent loss of phosphorescence intensity were minimized.

Table 4 summarizes the analytical figures of merit for anthracene on Whatman No. 1 filter paper pretreated with $TlNO_3$ and TlLS. The linear

Table 4. Room Temperature Phosphorescence of Anthracene in the Presence of 0.25 M $TlNO_3$ and 0.033 M TlLS

	LDR[a] (ng)	Slope (log-log)	Correlation Coefficient	LOD[b] (ng)	Precision[c] Method (%)
$TlNO_3$	3-75	1.16	0.998	3.5	8.34
TlLS	3-75	0.97	0.996	2.9	3.8

[a]Linear dynamic range (LDR) developed from analytical curve for anthracene on Whatman No. 1 filter paper pretreated with heavy atom. Measurements made in triplicate.
[b]Limit of Detection (LOD) calculated by 3 times the standard deviation of the blank divided by the linear slope.
[c]Precision for the method was obtained by the following formula:

$$P_{method} = \sqrt{\frac{s_{A+B}^2 + s_B^2}{I_{A-B}}} \times 100$$

Sixteen determinations of 10 ppm analyte and their respective blanks were utilized to determine; s_{A+B}, standard deviation of analyte + blank intensity; s_B, standard deviation of blank RTP intensity, and I_{A-B}, average net analyte RTP intensity.

dynamic range (LDR) of the calibration curves with either form of Tl(I) was 3-75 ng and the limit of detection (LOD) was approximately 3 ng. The linearity of the calibration curve on $TlNO_3$ was not as good as that produced on TlLS. The precision for the measurement of anthracene on filter paper treated with TlLS was observed to be 2 times better than that observed for the $TlNO_3$ system. These differences can be attributed to minimal loss of the anthracene phosphorescence intensity with irradiation time when using the TlLS system.

Conclusions

This comparative study of $TlNO_3$ and TlLS used as matrix modifiers for Whatman No. 1 filter paper that were used in the RTP analysis of anthracene, indicates that several advantages are incurred by the use of the surfactant, TlLS. The optimum heavy atom concentration investigations showed that a lesser amount of TlLS than $TlNO_3$ was required to produce twice the net signal intensity for anthracene. This is important in that less toxic metal is required for analysis. The observed enhanced signal intensity of anthracene with TlLS may be the result of several factors involving the lauryl sulfate anion. The large size and possible interactions of the sulfate anion may hold the heavy atom, Tl(I), closer to the surface of the filter paper. This is significant in that the closer the distance between the heavy atom and analyte, the better the stability of the triplet state and the greater the phosphorescence emission. Enhanced phosphorescence emission translates into a lower concentration of heavy atom required for analysis. Also, the alkyl nature of the anion should be resistant to atmospheric moisture which causes quenching of the analyte signal. This very condition should also be more

attractive for analysis of nonpolar and slightly polar organic compounds. In addition, these long alkyl chains of the lauryl sulfate anion appear to offer protection for the heavy atom and possibly for the analyte from photochemical reactions caused by source irradiation as evidenced by the RTP signal degradation investigations. One other possible explanation of the observed phosphorescence signal enhancement of anthracene produced by TlLS is that the long alkyl chains of the anion may entrap the analyte molecules, thus restricting their internal motions and restricting molecular collisions, both processes which decrease nonradiative deactivation of the triplet state.

This investigation demonstrated that it is possible to quantitate anthracene, a weak phosphor, at the nanogram level on Whatman No. 1 filter paper in the presence of Tl(I). An alkyl environment for Tl(I), as provided by a surfactant, was advantageous for solid substrate RTP analysis of anthracene.

A General Method for Solid Substrate Room Temperature Phosphorescence Analysis of Organic Compounds

Introduction

RTP is a very sensitive and selective analytical technique that is useful for nanogram detection of numerous compounds [4,12]. Although RTP analysis is a simple technique in concept, slight changes in the experimental conditions for analytical determinations can significantly affect the results. Reports of RTP analyses on solid substrates are numerous in today's literature, so also are the variations in experimental conditions for each analysis [4,8,9,12]. The variety in the details for analyses makes analytical determinations by this method confusing and

sometimes difficult for the analyst. These factors in combination with the inherent problems of solid substrate RTP analysis can render the technique unattractive for routine applications.

Variations in the conditions for RTP analysis involves choice of solvents, pH, heavy atom enhancers, substrates, and temperature. Each condition can affect the analytical results for a particular analyte. Manipulation of one or more of these conditions is frequently necessary to induce RTP and can have serious consequences on the results of the analytical determinations.

Inherent problems of solid substrate RTP include quenching reactions, impurity interferences, low resolution for mixtures containing more than one phosphor, stray radiation, inner filter effects, and background emission of the sample matrix. Probably because of the availability of numerous kinds of filter papers with different physical properties and the fact that RTP is observed from a large number of compounds, filter paper has become the most commonly used solid substrate for RTP measurements. [72]. The background radiation of filter paper spans from 300 to 600 nm and is present in all filter paper substrates that support phosphorescence emission. The intensity of the background emission varies with the excitation energy. Unfortunately, the excitation wavelengths for most organic molecules are in the same region as the maximum excitation for the background. RTP is not observed in the absence of background emission from the substrate. Solid substrate RTP analysis of organic compounds may require the use of heavy atom to stabilize the triplet state. Inorganic salts containing atoms such as lead, thallium, iodide, and silver are frequently the source of the heavy atom. In

general, these types of salts are virtually insoluble in nonpolar solvents and alcohols. The concentration of heavy atom required for phosphorescence enhancement generally limits the choice of solvent to water. The problems with water and filter paper are well documented in the literature and were reviewed earlier in this manuscript.

Our initial work on the modification of Whatman No. 1 filter paper with TlLS to be used for the solid substrate RTP analysis of anthracene eliminated some of the problems that are associated with filter paper and RTP measurements. TlLS is a surfactant and is amphiphatic. That is, it consists of a polar head and a long nonpolar hydrocarbon tail. The polar end of the compound contains the heavy atom, Tl(I) which is required for RTP emission from PAHs. The thallium ion can be envisioned, as depicted in Figure 9, as the anchor for this molecule on filter paper. The electrostatic attraction of the anionic sulfate groups for the thallium cation reinforces the anchor and leaves the long nonpolar alkyl chains to form an entangled network capable of entrapping phosphors. This entrapment can restrict the molecular motions of the molecules and increase the proximity of the heavy atom to the analyte molecule which can lead to enhanced phosphorescence emission. The alkyl chains should be resistant to the atmospheric water molecules and can serve to protect both the heavy atom and analyte molecule from source irradiation damage. The previously observed stability to heating and ultraviolet radiation, as well as phosphorescence signal enhancement, made TlLS the choice for matrix modification of filter paper.

This work is concerned with the investigation of Whatman No. 1 filter paper modified with TlLS as a possible general substrate that could

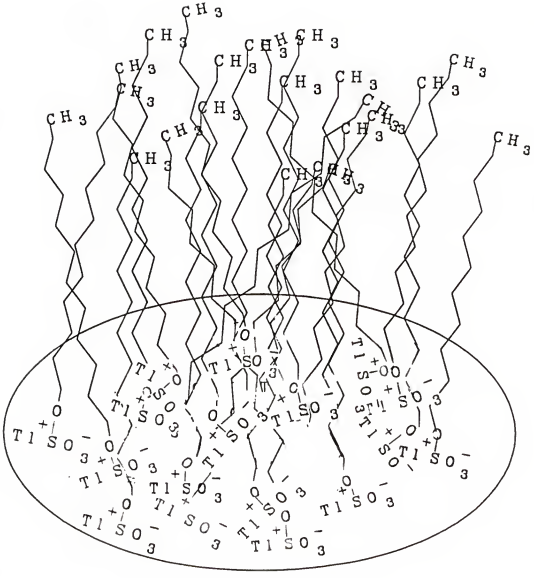


Figure 9. Filter paper disk modified with thallium lauryl sulfate.

be used for RTP analytical determinations, that involve minimal change in experimental parameters, for a variety of compounds. Whatman No. 1 filter paper, the most commonly used support for solid substrate RTP analysis, was modified with TlLS. The optimum heavy atom concentration of Tl(I) was determined and trace level detection of the analytes was accomplished. Analytical figures of merit have been presented and the limits of detection obtained have been compared to previously reported literature values. The effects of the background emission, i.e. stability and reproducibility, were addressed and optimization of the experimental parameters has been investigated. The emphasis of this investigation was on enhanced precision and simplicity of experimental procedures so that solid substrate RTP would be more useful for routine use.

Results and Discussion

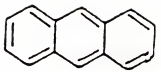
Whatman No. 1 filter paper pretreated with 0.065 M TlLS, Figure 5, produced a broad band of background emission from 300 to 600 nm. The excitation and emission maxima were 253 and 504, respectively. The RTP emission intensity is approximately 10 times that of untreated Whatman No. 1 filter paper under identical experimental conditions. The effect of the high background emission on the measurement of various PAHs, carbazole, and para-aminobenzoic acid (PABA) was investigated.

Table 5 lists the analytes and their RTP excitation and emission characteristics chosen for examination on Whatman No. 1 filter paper pretreated with TlLS, hereafter referred to as the substrate. Their structures are shown in Figure 10. These compounds were chosen because their emission maxima occurred in the same region as the background emission of the substrate and because of the similarities in their optimum

Table 5. Room Temperature Phosphorescence Spectral Properties of Several PAHs and PABA Examined on Whatman No. 1 Filter Paper Pretreated with TlLS.

Compound	TlLS (M)	λ_{Ex}(nm)	λ_{Em}[a] (nm)
Naphthalene	0.065 M	275	480, 513, (547)
Pyrene	0.065 M	336	593, 653
1,2,3,4-DBA	0.065 M	292	567, 615, 670
Carbazole	0.033 M	295	416, 440, (465)
PABA	0.033 M	286	430

[a]Wavelength of maximum RTP emission is underlined when there is more than 1 band; the wavelengths of the shoulders are in parenthesis; the precision for the wavelengths is ± 0.5 nm.



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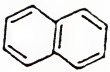

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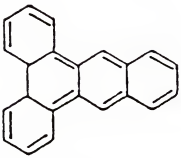

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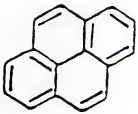
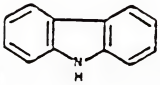
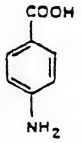


(d)



(e)



(f)

Figure 10. Structures of molecules examined on Whatman No. 1 filter paper pretreated with TlLS; (a) anthracene; (b) naphthalene; (c) 1,2,3,4-DBA; (d) pyrene; (e) carbazole; (f) para-aminobenzoic acid.

excitation wavelengths. Naphthalene was specifically chosen because of difficulties reported in the literature [94,102]. Naphthalene is suspected of volatilization by source irradiation in the sample compartment and therefore according to this author's knowledge, analytical figures of merit have not been reported. If indeed this does occur, the alkyl chains of TlLS might offer protection for the analyte. Carbazole and PABA were chosen because of their different polarities, in order to evaluate their interactions with the alkyl chains of the substrate.

PABA, Carbazole, naphthalene, 1,2,3,4-dibenzanthracene (1,2,3,4-DBA), and pyrene phosphorescence emission spectra were observed as broad bands with maxima at 430, 440, 513, 567, and 593 nm, respectively. The relative emission maxima represent different situations for the measurement of these compounds. Since the naphthalene emission maximum at 513 nm is very near the RTP emission maximum of the substrate, its analytical figures of merit should be the most adversely affected. Conversely, the other compounds should produce more favorable analytical results.

The optimum heavy atom concentration for each analyte was determined from a plot of the ratio (A/B) of the net analyte RTP signal intensity (A) to the substrate background RTP emission intensity (B) at the maximum wavelengths of the particular analyte versus the molar concentration of TlLS. This type of plot was necessary because of the large increase in background emission intensity observed for the substrate with increased concentration of TlLS. From Figure 11, it can be seen that the A/B ratios for all the analytes changed little with TlLS concentration. However, for all further studies, we chose the TlLS concentrations given in Table 5 for

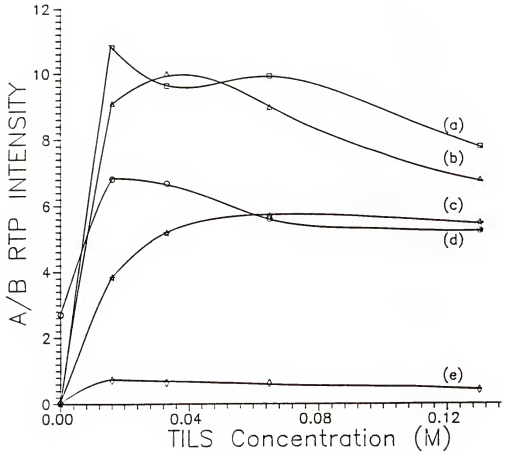


Figure 11. The ratio (A/B) of net analyte RTP signal intensity to the substrate background RTP emission intensity at the wavelengths of the particular analyte plotted versus the molar concentration of TlLS to determine the effect of heavy atom concentration on RTP emission of various PAHs; (a) 1,2,3,4-DBA; (b) carbazole; (c) pyrene; (d) PABA; (e) naphthalene.

the five analytes to achieve the maximal signal to background ratio. It should be noted that for untreated filter paper, PABA produced weak RTP emission intensity, in contrast to the other analytes tested, for which no phosphorescence emission was observed.

The reproducibilities of the RTP emission intensities for the pretreated substrates of the five analytes are given in Table 6. The optimum experimental conditions of each particular analyte were utilized for the measurements, i.e., optimum TlLS concentration, time of nitrogen purge in the sample compartment, and maximum excitation and emission wavelengths. The optimum percent relative standard deviations of the RTP background emissions of the substrate for all analytes was observed to be less than 7%. The measurement of 16 Whatman No. 1 filter paper substrates without TlLS, at the naphthalene excitation and emission maxima, produced a percent relative standard deviation of 11%. This suggests that possibly the surface of the filter paper was either made to be more uniform by the TlLS or that this modifier prevented moisture in the atmosphere from wetting the cellulose, a process which would tend to quench the background signal. It is also observed from Table 6 that although the background emission intensity of the substrate differed significantly at the various excitation and emission wavelengths, the reproducibilities differed by less than 2.5%. The best precision for substrate emission was produced by 1,2,3,4-DBA which was separated by approximately 60 nm from the maximum emission of TlLS. The worst precision was observed for PABA which was approximately 75 nm from the maximum emission of TlLS. This is in contrast to what was expected. The naphthalene excitation and emission maxima should have presented the least favorable results. Generally, high

Table 6. Reproducibility of Blanks at ex/em Maxima for Analytes

Analyte[a]	Number of Substrates	Avg. Substrate I	RSD (%)
PABA	37	11.8 ± 0.7	6.1
Carbazole	18	13.0 ± 0.7	5.4
Naphthalene	41	47.9 ± 2.8	5.7
1,2,3,4-DBA	30	20.1 ± 0.8	3.9
Pyrene	18	6.9 ± 0.3	4.3

[a]Excitation and emission maxima reported in Table 1.

background emission intensity produced poor reproducibility. At any rate, the differences observed for reproducibilities of the pretreated substrate were not significant.

The substrate was also checked for long term stability. The RTP background emission of 4 filter paper discs of the same batch of substrates used for the analysis of naphthalene was measured at the naphthalene excitation and emission wavelengths at approximately a 1 month interval. There were no significant differences in the reproducibilities of the RTP background intensity observed over the monthly period as opposed to the reproducibilities obtained over a few hours.

The analytical calibration curves for the analytes are presented in Figures 12, 13, and 14 and the AFOMS are summarized in Table 7. The slopes of the log-log calibration plots are all close to unity (± 0.03) with excellent correlation coefficients. Pyrene, carbazole, naphthalene and PABA show linearity over 2 decades, while 1,2,3,4-DBA was linear over only 1 decade. Nanogram detection limits were observed for all analytes. The precision of the method, defined in Table 4, for each analyte was determined using the 10 μg/ml standard solution for each compound. With the exception of naphthalene, precision for all analytes was observed to be less than 8% which is essentially the same as the precision of the background emission. The poorer precision (13.9%) for naphthalene is addressed in Table 8. This value for naphthalene is a little high and can be attributed to the high standard deviation of the substrate emission and to the low net analyte signal intensity. A decrease in the standard deviation of the substrate emission or an increase in the analyte signal intensity could produce more favorable results, assuming the chemical

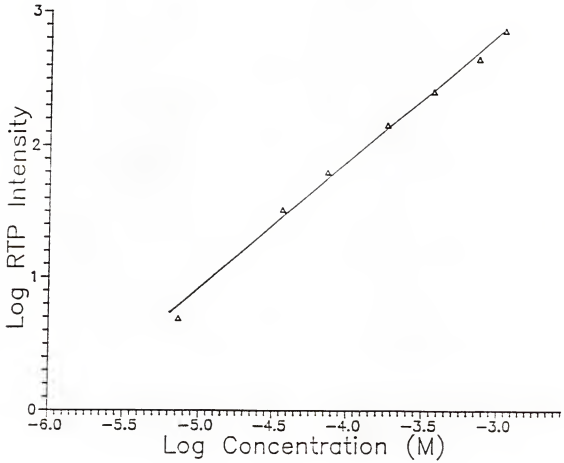


Figure 12. Calibration curve of log (Relative RTP Intensity) vs log (Analyte Concentration) of PABA on Whatman No. 1 filter paper pretreated with TlLS.

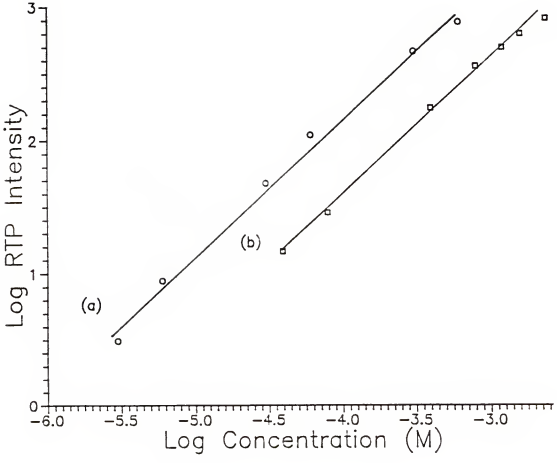


Figure 13. Calibration curve of log (relative RTP Intensities) vs log (Analyte Concentrations) on Whatman No. 1 filter paper pretreated with TlLS; (a) naphthalene; (b) carbazole.

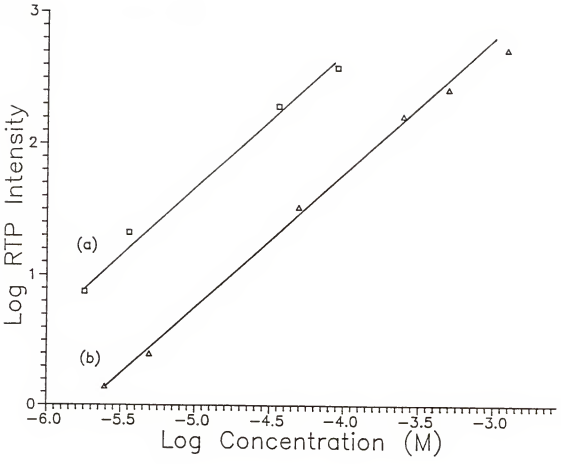


Figure 14. Calibration curves of log (Relative RTP Intensities) vs log (Analyte Concentrations) on Whatman No. 1 filter paper pretreated with TlLS; (a) 1,2,3,4-DBA; (b) pyrene.

Table 7. Rooom Temperature Phosphorescence of Organic Compounds on Whatman No. 1 Pretreated with TlLS

Compound	LDR[a] (ng)	Slope log-log	Correlation Coefficient	LOD[b] (ng)	Precision[c] Method (%)
PABA	3-450	0.98	0.997	1.3	7.6
Carbazole	1.5-300	0.99	0.996	0.8	7.8
Naphthalene	15-450	1.02	0.998	8.8	13.9
1,2,3,4-DBA	1.5-75	1.03	0.996	0.5	5.8
Pyrene	1.5-750	0.97	0.998	1.4	3.3

[a]Linear dynamic range resulting from triplicate measurements of each analyte on Whatman No. 1 pretreated with TlLS.
[b]Limit of detection calculated by $3s_{blank}/m_{linear}$.
[c]Sixteen determinations of 10 ppm analyte and their respective blanks were utilized to determine the precision for the method. Formula in Table 4.

Table 8. Comparison of 16 Measurements of the Signal Intensity of 10 μg/ml Naphthalene with Their Respective Substrates.

	Avg. RTP Intensity	RSD
Avg. Substrate[a]	47.4 ± 2.7	5.7%
Substrate + Naphthalene	76.2 ± 3.0	3.9%
Naphthalene	28.8 ± 4.0	13.9%

[a]The substrate is Whatman No. 1 pretreated with 0.065 M TlLS.

properties of the analyte were well-behaved. It should also be mentioned that the time for the naphthalene RTP signal to stabilize was more than twice that of the other compounds tested. The RTP intensity peaked at a maximum value after 10 minutes of drying in the sample compartment with dry nitrogen, and then rapidly degraded. Suspecting that volatilization of the analyte during the drying time might be responsible for the unfavorable precision observed, a new experiment was done.

Whatman No. 1 filter paper was pretreated with a saturated solution of TlLS in ethanol/water (80:20 by volume) and utilized for the analytical determination of naphthalene. It was hoped that by packing the cellulose matrix more densely with the surfactant, the analyte molecules would have greater protection from the source irradiation. Table 9 compares the results of this study with the previous work using the 0.065 M TlLS. Calibration curves for both concentrations of TlLS have excellent linearity and excellent correlation coefficients. The substrate emission intensity of the support with high TlLS concentration is decreased to half the value observed from the 0.065 M TlLS substrate. The standard deviation of the blank emission intensity is also less, thus the calculated limit of detection is more favorable. In addition, the precision of the method is now observed to be 2 times better than that observed in the previous investigation. Another observation is that the LDR is one-third of that produced by the lower concentration of TlLS. This same phenomena of a decrease in the background emission intensity of the substrate with very high heavy atom concentration was observed on the heavy atom curve for anthracene and $TlNO_3$ but was not considered significant because the background emission intensities were very small

Table 9. The Effect of Increased TlLS Concentration on the Analytical Figures of Merit for Naphthalene

TlLS (M)	LDR (ng)	Slope log-log	Correlation Coefficient	LOD (ng)	Precision Method
0.065	15-900	1.02	0.998	8.8	13.9
0.13	15-300	0.986	0.999	4.0	6.05

compared to this case. A possible explanation for the decrease in background emission intensity of the substrate with high concentration of surfactant could be explained by the dense entangled outer atmosphere of the alkyl chains.

The alkyl coating on the surface of the filter paper might possibly shield the cellulose substrate and the Tl(I) atoms from source irradiation which would decrease the emission intensity of the background. This is unlikely since the alkyl environment is missing from the substrates pretreated with $TlNO_3$. The observation of decreased emission intensity of the substrate with high heavy atom concentrations is probably due to inner filter effects which are known to occur at high concentrations of absorbing species. The analyte might be shielded by entanglement in the long chains from direct and constant irradiation of the source which could result in volatilization, but this does not appear to be the case in these investigations. Examination of Table 7 with the latter investigation of naphthalene at high TlLS concentration confirms that the poorer reproducibility observed for naphthalene relative to the other analytes is attributed soley to the high standard deviation of the substrate background emission. Volatilization of naphthalene does not occur with the experimental conditions used in these analysis, but could be a problem if source irradiation time was not optimized. One other point is that the net signal intensities, for the same concentration of analyte, observed for 0.065 M and 0.13 TlLS were nearly identical. Enhancement of analyte signal intensity with increased heavy atom concentration was observed only for high naphthalene concentrations at 0.065 M TlLS. At high

concentration of analyte, quenching occurred in the presence of high TlLS concentration.

Conclusions

TlLS was used as a matrix modifier for Whatman No. 1 filter paper. This new substrate was used successfully for nanogram detection of several organic compounds with minimal changes in the experimental parameters, i.e., time of nitrogen purge for drying of analyte solvent while in the sample compartment. The LODs compare favorably with previous literature reports. Table 10 lists the variety of filter papers and matrix modifiers utilized for the analyses of these molecules. Other variations in the experimental conditions can be found in the references listed. Naphthalene, a difficult phosphor for RTP determination, was measured successfully and the analytical figures of merit reported.

This study suggests that Whatman No. 1 filter paper pretreated with TlLS can be used as a general method for trace analysis of various kinds of compounds. The substrate was stable over a long period of time, meaning it could be prepared ahead of time and stored for future use. The reproducibility of the substrates background was less than 7%. All of the analyte signals, with the exception of naphthalene, reached a steady state in less than 3 minutes as long as a nitrogen purge was used. A more densely packed filter paper matrix, produced more favorable results for the analysis of naphthalene. Although PABA and carbazole were chosen because of their polarity differences with respect to the PAHs, these species were just as well behaved as the nonpolar entities.

Table 10. Literature Survey of LODs for the Organic Compounds Examined on Various Matrix Modified Filter Paper Substrates Compared Those Obtained on Whatman No. 1 Filter Paper Pretreated With TlLS.

Analyte	Filter Paper	Matrix Modifier	LOD (ng)	Ref.
PABA	Eaton Dikeman 613	NaOH	0.048	17
	Eaton Dikeman 613	NaOH:NaI	0.062	17
	Schleicher & Schuell 591-C	NaOH:NaI	0.1	94
	Whatman No. 1	TlLS	1.3	
Carbazole	Schleicher & Schuell 2040-A	CsI	0.005	64
	Whatman No. 1	$TlNO_3$	8.2	92
	Eaton Dikeman 513	NaOH:NaI	0.7	62
	Whatman No. 1	TlLS	0.8	
1,2,3,4-DBA	Schleicher & Schuell 2040-A	CsI	0.08	64
	Whatman No. 1	TlLS	0.5	
Pyrene	Schleicher & Schuell 2040-A	$Pb(OAc)_2$	0.1	64
	Schleicher & Schuell 591-C	---	2.7	94
	Whatman No. 1	$TlNO_3$	0.5	92
	Whatman No. 1	TlLS	1.4	
Naphthalene	Whatman No. 1	TlLS	8.8	
Anthracene	Whatman No. 1	TlLS	2.9	
		$TlNO_3$	3.5	

Experimental Conditions

Apparatus

The RTP spectra and intensity measurements were collected with the Perkin-Elmer LS-5 luminescence spectrofluorometer (Perkin-Elmer, Norwalk, CT) interfaced to a model CLS-3600 data station as described in Chapter 2. All spectra were collected in the phosphorescence mode using a delay time of 0.03 ms and a gate time for collection of data of 9.0 ms. The excitation and emission slits were set at 10 and 5 nm, respectively, for all compounds with the exception of anthracene. Anthracene was measured at slit widths of 5 and 10 nm, respectively. A 360 nm cut-off filter was used in front of the emission monochromator in order to minimize second order scatter when necessary. The scanning of excitation and emission spectra at scan rates of 120 nm/min and the resulting peak intensity and wavelength were processor controlled by the Perkin-Elmer PECLS application program.

Aluminum sample holders were constructed to fit the multiple sampling cuvette holder for the spectrofluorometer [95]. The area of sample irradiated by the source was approximately 1 cm; therefore a 1 cm circle was cut into the face plate of the sample holder. The filter paper support was placed between the face plate and the body of the modified cuvette. Care was taken to insure the face plate fit snuggly against the body of the cuvette. Since the preparation of the modified support required extended heating, there was a tendency for the paper to curl. It was necessary for the face plate of the sample holder to hold the support firm and flat for good reproducibility. Also, the best precision

was obtained when using the same sample holder and the same position of the cuvette holder in the spectrofluorometer.

Reagents

Thallium nitrate and Naphthalene (Mallinckrodt, St. Louis, MO), 4-aminobenzoic acid (PABA) and carbazole (Eastman Kodak Co., Rochester, NY), 1,2,3,4-dibenzanthracene (1,2,3,4-DBA), anthracene, and pyrene, (Aldrich Chemical Co., Milwaukee, WI) were reagent grade and used as received. The PABA was used as a reference compound for comparison of RTP figures of merit. Whatman No. 1 filter paper was cut by special order into disks 12 mm in diameter and purchased from Whatman Chemical Separation Inc., (Clifton, NJ). Thallium lauryl sulftate (TlLS) was prepared in our lab. See procedures. Absolute ethanol (Florida Distillers Co., Lake Alfred, FL) and "nanopure" demineralized water (Barnstead System, Sybron Corp., Boston, MA) were used as solvents.

Procedures

The following procedures were used for all investigations included in Chapter 3 unless otherwise noted in the text.

Preparation of TlLS. TlLS was prepared by a procedure similar to Humphrey-Baker, et al. [86]. Aqueous solutions of 0.25 M sodium lauryl sulfate (NaLS) and 0.25 M $TlNO_3$ were prepared. A 250 ml aliquot of the NaLS solution was mixed with 500 ml of $TlNO_3$ solution. The mixture was stirred and gently warmed for approximately 2 hr. Removal from the heat and subsequent cooling of the mixture to room temperature initiated formation of the white precipitate, TlLS. The precipitate was collected after 10 hours, isolated by gravity filtration, washed with deionized water, and allowed to air dry for 24 hr. TlLS was recrystallized from

water, until the melting point of the TlLS became constant at 197°C. A 60 percent yield of colorless crystalline product was obtained.

Preparation of the Paper Supports. Whatman No. 1 was purchased as 1.2 cm filter paper disks. Batches of these disks were placed in petri dishes and each filter paper was spotted in the center with 5 μl of the appropriate heavy atom solution. The disks were allowed to air dry for approximately 2 min, placed in an oven and heated to 110°C under a continuous flow of nitrogen for 3 hr, and stored in a dessicator for a minimum of 24 hr before use.

Preparation of Solutions. The heavy atom solutions of $TlNO_3$ were prepared in water and those of TlLS were prepared in ethanol/water (80: 20 by volume). All analyte solutions were prepared daily in absolute ethanol by serial dilution of 500 or 1000 μg/ml, depending on the respective solubilities of the analytes.

Measurement Procedures. The prepared substrate, i.e., Whatman No. 1 treated with 5 μl of the appropriate concentration of heavy atom, was placed in the sample holder and dried under nitrogen for approximately 5 min. The RTP signal intensity was determined at the maximum excitation and emission wavelengths for each analyte. A 3 μl sample of analyte was spotted onto the center of the measured support. It was allowed to air dry for 2 min and then placed in the sample compartment for drying under nitrogen for approximately 5 min. The emission intensity of the analyte was recorded at the appropriate maxima excitation and emission wavelengths for that analyte. Each sample had its own blank whose intensity was subtracted from the total intensity measured for the analyte. All measurements were performed in triplicate in a nitrogen atmosphere.

CHAPTER 4

LUMINESCENCE BY ENERGY TRANSFER IN SOLUTION

Background

Absorption of radiation by molecules in solution can result in electronically excited states. Ultimately, deexcitation of the excited state occurs through the emission of light or heat. In the time interval the molecules are in the excited state, prior to deexcitation, the absorbed energy can relocate to another area of the molecule or to different molecules in the system. The molecules excited originally are generally designated as the donors, while the latter excited molecules are referred to as the acceptors. This movement of energy between donor and acceptor molecular species is referred to as energy transfer and is known to be a very efficient process [56,67,103]. If the energy movement is within the same molecule, it is termed intramolecular energy transfer, while the relocation of energy to a different molecular species is referred to as intermolecular energy transfer. Either process generally results in quenching of the donor species and may or may not result in luminescence characteristic of the acceptor species. Because these phenomena occur in many kinds of materials in different physical states and under various experimental conditions, the investigation and exploitation of these processes are of interest to researchers in several scientific areas [3,4,67]. Major applications of energy transfer in

chemical analyses are focused on biological applications, while fundamental studies have been favored by the physical chemist.

Energy Transfer Theory

Energy can be transferred between molecules by radiative and nonradiative processes [3]. Both processes require overlap of the emission spectra of the donor with the absorption spectra of an acceptor. A radiative energy transfer process involves the radiative emission from a donor molecule in the excited singlet state and the absorption of that same emission by an acceptor molecule in the ground singlet state. This process describes sensitized fluorescence, or singlet-singlet energy transfer, that may be observed in solution. Radiative processes of singlet-singlet energy transfer are termed "trivial" processes and are considered the less important of energy transfer phenomena that occur in solution [3,104]. Phosphorescence is rarely observed in solution and consequently, radiative energy transfer involving the triplet excited state is not normally observed.

Nonradiative energy transfer processes in solution occur with no observable intermediate emission or absorption. Energy transfer from a donor to an acceptor molecule can occur by one of two processes, both of which depend on the concentration of the acceptor species. These two processes are referred to as the resonance excitation or the dipole-dipole mechanism and the exchange mechanism which is diffusion controlled [4,104,105].

The development of the mechanism for resonance excitation energy transfer is attributed to Förster [67,106]. By this mechanism, energy transfer proceeds by a radiationless process in which the initial excited

molecules transmit the excitation energy to acceptor molecules over some distance. Initially, the donor molecule absorbs radiation and is excited to a higher vibrational level of its electronic excited state. Vibrational relaxation to the lowest vibrational level of the first excited electronic state occurs quickly. In the event there is an acceptor species nearby that has an absorption transition equal in energy to the deactivating process of the donor, both processes may occur simultaneously. The result is that there is a nonradiative transfer of energy from the donor to the acceptor species. The resonance energy mechanism implies that the energies may be smaller than that of the donor, but any difference must be accounted for by the vibrational levels in both the acceptor and donor molecules. An important criterion for transfer of energy by this mechanism is the overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor. The greater the overlap, the more probable that energy transfer will occur. A major point of this mechanism is that the donor and acceptor molecules are not in contact with one another and that excitation energy is transferred over distance. Energy transfer by this process has been observed to occur over distances of 10^{-6} and 10^{-7} cm. This mechanism as described by Förster [106] is based on the assumption that the interaction energy between the donor and acceptor molecules is small in comparison to the width of the vibronic bands of the excited states and that vibrational relaxation is a fast process in comparison to the excitation transfer rate. The nature of interactions between the donor and acceptor are resonant or dipole-dipole. According to Förster, the rate constant of resonance energy

transfer is a function of distance and is given by the following equation [105].

$$(17)\quad k_{ET}\ (D^* \rightarrow A) = \left(\frac{1}{\tau_D}\right)\left(\frac{R_o}{R}\right)^6$$

where

k_{ET} = rate constant for energy transfer from excited donor to excited acceptor; in $mol^{-1}\ sec^{-1}$;

τ_D = actual mean lifetime of excited donor, in s;

R_o = critical separation between donor and acceptor for which energy transfer from the excited donor to the acceptor and emission from the excited donor are equally probable; in Å; and

R = actual separation between the centers of the excited donor and the acceptor molecules, in Å.

The critical separation, R_o, can be determined from the following equation:

$$(18)\quad R_o = \left(\frac{3000}{4\pi\ N\ [A]_{\frac{1}{2}}}\right)^{1/3}$$

where

N = Avogadro's number; and

$[A]_{\frac{1}{2}}$ = concentration of acceptor molecules that reduces the quantum yield of fluorescence of the donor to half of its value measured in the absence of the acceptor species, in mol l^{-1}.

The efficiency of energy transfer, is given by equation 19 and can be easily determined experimentally by equation 20.

$$(19)\quad \phi_{ET} = \left[1 + \left(\frac{R}{R_o}\right)^{6}\right]^{-1}$$

$$(20)\quad \phi_{ET} = 1 - \left(\frac{I}{I_o}\right) = 1 - \left(\frac{\tau}{\tau_o}\right)$$

where

I = intensity of donor luminescence in the presence of the acceptor;

I_o = intensity of the donor luminescence in the absence of the acceptor;

τ = donor lifetime in the presence of the acceptor, in s; and

τ_o = donor lifetime in the absence of the acceptor, in s.

It can been seen from these two equations that R_o and $[A]_{1/2}$ are the important factors for the donor-acceptor pair and resonance energy transfer. High concentrations of the acceptor species mean that the critical separation between the donor and acceptor dipoles is small and the probability of resonance energy transfer is more favorable. The rate of resonance energy transfer depends not only on the critical separation and acceptor concentration, but on the lifetime of the donor's excited state and the molar absorptivity of the electronic transitions in the acceptor species. Resonance energy transfer is not controlled by the rate of diffusion. The lifetime of the excited single state and the rate of diffusion are long compared to the time for occurrence of energy transfer and the donor and acceptor molecules are not required to be in contact with each other. Singlet-singlet and singlet-triplet excitation of

acceptors by excited singlet donors is commonly observed in resonance energy transfer systems because of the high probability of singlet-singlet transitions in both acceptor and donor molecules. This high probability of the transitions compensates for short lifetimes of singlet excited states. The long lifetime of singlet-triplet donors does not compensate for the low probabilities of the transition; therefore, both singlet and triplet sensitized phosphorescence are not generally observed by the resonance energy transfer process.

The theory for resonance energy transfer was developed by Förster to explain energy transfer between molecules over long distances [106]. Dexter utilized the Förster equations and extended this theory to explain energy transfer between donor and acceptor molecules that were in close contact, molecules that would be no further apart than their collision diameters. The Dexter work provided the exchange mechanism of energy transfer [107].

Singlet and triplet sensitized phosphorescence observed in solution are explained by the exchange mechanism of excitation energy transfer [4,107]. In both cases, the excited singlet or excited triplet of the donor molecule must be higher in energy than the triplet excited state of the acceptor molecule. The most common event observed by this mechanism is triplet-triplet energy transfer probably because the singlet-triplet transition of the acceptor is of low probability. This type of energy transfer occurs when the donor and acceptor molecule are close enough so that their electron clouds can overlap; therefore, this is a short-range phenomena. In the region of overlap of the electron clouds, the electrons become indistinguishable and therefore, the excited electron on a donor

molecule can easily be exchanged for another of equal energy on the acceptor molecule. Because of the close contact of the donor and acceptor molecules, this type of energy transfer is diffusion limited. Singlet-singlet energy transfer in solution is also allowed by the exchange mechanism. Enhanced luminescence and quenching of the lanthanide chelates in solution are both explained by exchange energy transfer.

Applications Involving Energy Transfer and Lanthanides

Current interest in rare earth chemistry is centered on the preparation and utility of lanthanide complexes. Although little is known about the chemistry of the lanthanides relative to other groups in the periodic table, such as the alkaline rare earths and transition metals, the popularity of lanthanide complexes as shift reagents for nuclear magnetic resonance (NMR) spectroscopy has prompted numerous fundamental studies on the chemistry and physical properties of the rare earths. Current use of lanthanide complexes include their utility as metal centered probes for structural studies of biological macromolecules and polymeric materials [108-111]. In addition, application of trivalent lanthanide chelates as luminescence markers for nonradioisotopic immunoassays is a rapidly growing area for medicinal chemistry [112-116].

The majority of metal ions that are known to be important in biological structure and function do not luminesce in aqueous solution [117]. Also, the number of metal ions and their complexes, including most of the transition metal ions, that have been used as absorbance or magnetic resonance probes for biomolecular systems are not luminescent in solution media at room temperature. Frequently, these metal ions act as quenchers when bound close to lumiphors. Europium is one of the few

exceptions to the nonluminescent behavior of metal ions. Trivalent europium, Eu(III), is probably the most studied of the lanthanide metals principally, because it is known to form highly fluorescent chelates with numerous as well as varied, organic ligands.

Physical and Chemical Characteristics of Europium

It is the f-orbitals and the electrons occupying them that distinguishes europium and the other lanthanides from other chemical groups in the periodic table [118-120]. The electron configuration for the ground state of europium is $[Xe]4f^{7}6s^{2}$. The outermost electronic configuration presents a completely paired, noble gas type of electronic configuration. The lowest lying excited states of europium and its complexes are comprised of the 4f electronic configurations. The 4f electrons are lodged in the third shell from the outside, meaning they are shielded from the exterior environment. For this reason, the f-orbitals and their electrons are unavailable for coordination or bond formation. Covalent bond formation therefore, results from the higher energy 5d orbitals. The most stable oxidized state of europium is the 3+ state, although a few cases of 4+ and 2+ oxidized states have been reported [119]. Trivalent europium is formed by the loss of the two 6s electrons and one of the 4f electrons. The resulting cation is large in size, thus causing the charge to ionic radius ratio (ionic potential) to be low. This condition is responsible for minimized electrostatic interactions with ligands, as well as weak bonding interactions. As a result, there is no directionality in the europium-ligand bonds so that primary coordination numbers and geometry of the complex is determined solely by the geometry and bonding characteristics of the ligand. Europium

complexes are spherical in shape and the results of previous investigations involving synthesis, and ion exchange and solvent extraction processes, suggest ligand exchange reactions in solution are extremely rapid [120]. It has also been observed in synthesis investigations that complexes existing in solution can be difficult to isolate in the solid state. Also once the complex has been obtained in the solid state, there are frequently problems with redissolving it.

In aqueous solution, europium complexes are described as bulky spherical structures consisting of two different regions [118,121,122]. The primary or "inner" coordination sphere consists of molecules of water that are in the direct vicinity of the europium ion. These water molecules have been differentiated from the molecules in the bulk solvent, by kinetic experiments. The number of water molecules in this coordination sphere dictates the coordination number for europium. In general, coordination numbers for complexes are defined with respect to a crystal lattice. Because of the lability of ligands with the lanthanide cations, including europium and the difficulties of isolating complexes in the solid state, several investigators have used x-ray scattering and fluorescence lifetime techniques for estimating the coordination numbers of europium in aqueous solution [121]. These coordination numbers, termed hydration numbers, are considered estimates and the hydration number for europium has been estimated as 8 or 9. Beyond the primary coordination sphere of water molecules is a second sphere, or outer shell, containing additional water molecules that are not in direct contact with the metal ion but which interact with the water molecules in the inner sphere.

These water molecules are also different from the water molecules of the bulk solvent.

Europium, as well as the other lanthanides, is identified as a type "a" cation according to the Ahrland, Chatt, Davies classification scheme or as a hard acid as described by the Pearson model [117,118]. This means that the metal ion has a preference for negatively charged donor groups, such as the carboxylate anions. Molecules containing negatively charged oxygen atoms are the strongest binding ligands for europium. The binding preference for donor atoms follows the trend O > N > S. Water molecules and hydroxide ions are strong ligands that compete for the binding sites on Eu(III) cations. In fact, in neutral or aqueous solutions, the only ligands that can successfully compete against water and hydroxide ion for the binding sites are restricted to molecules containing more than one donor group, i.e. chelates. Neutral oxygen and nitrogen atoms will occupy binding sites on the metal cation only if they are constituents of multidentate ligands containing at least one other donor group that is a negatively charged oxygen atom. In basic solution, the hydroxide is a stronger competitor for the binding sites on europium than water molecules, thus yielding complex precipitation. Also, the addition of a nitrogen donor ligand to a neutral aqueous solution of europium can easily form sufficient concentration of hydroxide ion to cause precipitation of the metal ion. Because of the strong affinity of water and hydroxide moities for europium, the solvent media for the preparation and isolation of europium complexes is generally restricted to weak donor solvents [119].

Spectroscopy and Europium

The area of sensitized emission from lanthanide chelates containing organic ligands was first introduced by Weissman in 1942 [123]. Absorption spectra of these complexes exhibited a broad absorption band characteristic of the ligand portion of the complex. Excitation of the ligands of Eu(III) complexes of salicylaldehyde and β-diketonate ligands with ultraviolet light resulted in narrow emission bands characteristic of trivalent europium. It was also noted at this time that luminescence intensity increased with dissolution of the complexes in nonpolar solvents and also with a decrease in temperature. As noted, this was the first report of energy transfer for lanthanide complexes containing organic ligands. The pathway for energy transfer within the lanthanide complexes was later established by Crosby and coworkers [124,125]. Figure 15 is a schematic diagram of the path of energy migration in a Eu(III) complex in which the europium ion can be sensitized by organic ligand. Absorption of ultraviolet radiation by the chelated ligand results in an excited singlet state on the ligand. In this system, the europium ion can effectively function as a heavy atom, and promote ISC to the excited triplet state of the ligand. At least two processes can occur from this excited state. Since this discussion concerns aqueous media, the excited triplet state of the ligand can undergo nonradiational deexcitation to the ground state. Collisions between the ligand and solvent molecules destabilize excited triplets. Another process that can occur is that the excitation energy can be transferred to the metal ion by either the exchange of resonance energy transfer mechanisms. For the exchange process to occur, there must be overlap of the excited ligand triplet

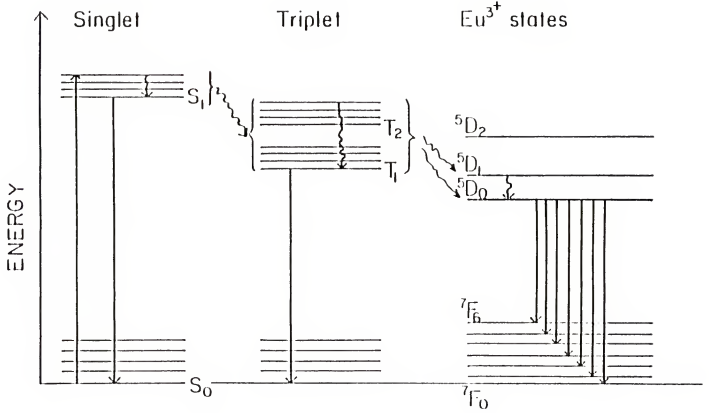
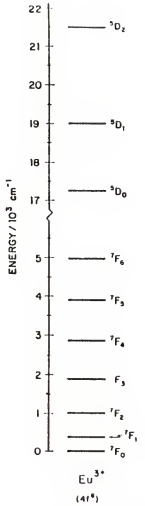


Figure 15. Schematic diagram of the path of energy migration in a Eu(III) complex in which Eu(III) emission can be sensitized by an organic ligand.

state with the D_0 or D_1 vibrational manifold of the lanthanide ion. For the resonance mechanism to be in effect, the energy of the excited ligand triplet state must be sufficient to be transferred to a resonance level within the 4f system of the metal ion. That is, the energy difference of deactivation from the triplet state of the ligand must correspond exactly or be only slightly greater than one of the absorption transitions of the europium ions [67]. Regardless of which mechanism is operative for the energy transfer, the subsequent emission of radiation occurs from the D_0 excited state to the F level of the metal ion. The luminescence emission is observed as narrow bands characteristic of the Eu(III) ion.

For aqueous solutions containing europium(III) complexes, essentially all emission is derived from the D_0 excited state as long as the excitation wavelengths are shorter than 578 nm. See Figure 16. The strongest emission is produced by transitions between D_0 and F_1 or F_2. The transition between D_0 and F_4 is frequently observed as having moderate intensity. All other transitions to the F_n levels are either very weak or unobservable. The strongest emission bands are susceptible to splitting and intensity changes which are due to the detailed nature of the ligand environment around the metal ion. The term for this effect is "hypersensitivity" [117,120]. It should also be noted that the absorption transitions of F_0 to D_0 can be hypersenstive. The information obtained from this latter transitions is useful for the determination of the presence of more than one europium species and the indication of more than one binding site on a ligand. The absorption and emission hypersensitivities usually require the use of a tunable dye laser for the excitation source. The hypersensitivity of the europium bands, as well



Transition	Spectral Region (nm)	Relative Intensity
$^5D_0 \rightarrow {}^7F_6$	~813	not observed
$\rightarrow {}^7F_5$	~750	not observed
$\rightarrow {}^7F_4$	680-705	medium
$\rightarrow {}^7F_3$	645-660	weak
$\rightarrow {}^7F_2$	610-630	strongest
$\rightarrow {}^7F_1$	585-600	strongest
$\rightarrow {}^7F_0$	578-580	weak

Figure 16. Energy level diagram for the lowest energy multiplet levels of Eu^{3+} ($4f^6$) and the major spectral characteristics of Eu^{3+} emission spectra for complexes in aqueous solution [117].

as those of other lanthanides, are currently exploited for determination of biological structures [117].

The technique of energy transfer is very useful for structure elucidation as well as for use as a luminescent tag for nonluminescent species. An intriguing use of energy transfer to increase the sensitivity of the luminescence analysis of tetracycline and various analogues by chelation to europium was reported by Hirschy and co-workers [126,127]. Later work by these same investigators demonstrated the potential usefulness of energy transfer in moving an analyte signal away from an interfering background [128].

The present work addresses the analysis of 1,3-dimethylxanthanine, a cyclic-diketone containing nitrogen heteroatoms in aqueous solution by energy transfer to europium(III). This investigation differed from the Hirschy work in that the analyte binds very weakly, if at all, to the metal ion. Also, the analyte does not normally luminesce in aqueous solution without perturbation by a heavy atom, and any luminescence that might occur would be obscured by the background of the sample matrix. The luminescence intensity o Eu(III), being far removed from background interference, was enhanced by the analyte. The methodology for analytical determination of 1,3-dimethylxanthine (theophylline) in aqueous solution by energy transfer was developed. This methodology was also applied to the successful analysis of this same analyte in a mixture containing 1,3,7-trimethylxanthine (caffeine) a known interferent.

Energy Transfer Between 1,3-dimethylxanthine and Europium (III) in Aqueous Solution

Introduction

Since the discovery that luminescence emission could occur at room temperatures, numerous investigators have utilized a variety of methods to induce phosphorescence and fluorescence from a diverse array of organic compounds [4,5,8,9,12,24,67]. A large number of these investigations have been centered on the use of solid substrates for analytical determinations since phosphorescence is not normally observed in solution [12,24]. The principal advantage of solid substrate analyses is that of small sample requirements. The principal disadvantage is the presence of water which tends to quench the luminescence signals of lumiphors. Although water is not as deleterious to fluorescence techniques as it is to phosphorescence analyses, it still causes serious reproducibility problems when cellulose substrates are utilized as the solid support. Cellulose substrates, i.e., filter paper, are the most common solid support used in solid substrate analysis today. In addition to problems associated with water, high background radiation of filter paper substrates is observed in the spectral region where many organic compounds luminesce, thus sensitivity as well as reproducibility can be affected. New trends in phosphorescence analysis are directed toward luminescence analyses in fluid solutions [4,5,12].

Fluorescence analysis in aqueous solution have been used for a long time [1,4,5,67]. The sensitivity of the analytical technique and applications to biological, medical, and pharmaceutical sciences have made this type of luminescence analyses a valuable tool for these scientific

areas [67]. Phosphorescence analyses in aqueous solutions offers additional selectivity to luminescence analyses. Unfortunately, the development of this technique has been severely handicapped by quenching processes in the liquid state. Development of phosphorescence methods for analytical investigations in aqueous solutions are important because of the potential applications to biological samples.

The determination of organic molecules by RTP in normal liquids is a relatively recent development in luminescence analysis [59,129-132]. Donnerbroek and co-workers have used biacetyl for sensitization and quenching RTP from organic and inorganic analytes. Biacetyl is an exception to the fact that organic molecules do not phosphoresce in solution under normal conditions. These investigators evaluated common solvents, hexane, methanol, acetonitrile, water, and dichloromethane, as suitable media for sensitization of selected PAHs. Acetonitrile was their solvent of choice for the analytical determinations. Several recent investigations by Cline-Love and co-workers involved the use of micelles, cyclodextrins and colloidal suspensions for analytical determination of solution luminescence of organic analytes [87,132-135]. They reported significant luminescence signal enhancements for several compounds in the presence of micellar solutions. All of these methods involve the direct irradiation of the analyte and the subsequent measurement of it's emissive luminescence. Current developments for luminescence methods in aqueous solution involve the concept of energy transfer to rare earth ions [117-120].

The energy transfer phenomena was first observed over 40 years ago and the theory explaining it followed 10 years later [106,107,123].

Energy transfer reactions occupy very important roles in a variety of physical, chemical, and biological systems and consequently, investigations involving these phenomena are numerous [67,103-105,109,117,118,120]. A currently popular research area involves energy transfer reactions between analytes and lanthanide ions. Trivalent europium and terbium ions are the most common of the rare earths to be studied because of their ability to luminesce in aqueous solutions and retain that luminescence when bound to macromolecules [117]. Both europium and terbium ions exhibit multiple emissions. The hypersensitivities and intensities of the emission bands depend on the nature of the ligand environment around the metal ion. This makes rare earth ions particularly attractive for use as spectroscopic probes in biological systems.

The chelation of Eu(III) and Tb(III) by a variety of organic ligands can result in sensitized luminescence of the rare earth ion. Excitation energy is absorbed by a ligand donor and transferred to the acceptor metal ion. Enhanced luminescent radiation is subsequently emitted that is characteristic of the rare earth ion. The metal ion luminescence is emitted as a narrow band with a large Stokes shift and an exceptionally long luminescence lifetime. Although many investigations of europium chelates have been reported in the literature, very few of these studies utilized water as the solvent. This is principally due to the physical and chemical characteristics of the europium ion itself.

Europium forms relatively few complexes in solution because the f-orbitals of the lanthanide ion are unavailable for covalent bond formation and the large ionic radius that is characteristic of all the lanthanides

tends to reduce electrostatic forces of attraction [118,119]. Eu(III) is classified as a "hard" acid in the Pearson classification scheme, therefore it is expected that it would prefer a "hard" base. It is well understood that the interactions between Eu(III) and ligands are electrostatic or ionic in nature and the binding preference is for negatively charged oxygen groups. This is the reason that the majority of previous investigative studies of europium chelates employed solvents such as acetic acid and acetonitrile, that were capable of ionizing the appropriate functional groups of the ligands [136]. There are some reported instances where the ligand itself served as the solvent [137-138].

In aqueous solution, the hydroxide ion and water molecules compete successfully for coordination sites of the europium ions. Therefore, only groups containing negatively charged oxygen atoms can compete. Neutral oxygen and nitrogen groups compete only if the ligand contains a negatively charged oxygen. Although there are several reports in the literature of energy transfer reactions using aqueous media, these mainly refer to studies of lanthanides as luminescence probes for structural studies or investigations of photophysical properties of lanthanides and energy transfer processes [117-120,126]. Quantitative applications appear to be limited. In the present investigation, 1,3-dimethylxanthine, a cyclic β-diketone is used as a model compound for the sensitized luminescence of Eu(III) ion in buffered aqueous solution. Excitation energy absorbed by this 1,3-dimethylxanthine was transferred to Eu(III) which luminesced in a region far removed from the interfering background radiation of the analyte matrix. The emission intensity of Eu(III) was

enhanced in proportion to the analyte concentration, thus permitting quantitative analysis. To this author's knowledge, analytical determination of this compound has not been previously reported in any sort of solution media. The methodology for the quantitative analysis of 1,3-dimethylxanthine has been determined and the analytical figures of merit presented. Problems associated with analysis by energy transfer are also addressed.

Results and Discussion

There are certain criteria that must be met for the observation of energy transfer between donor and acceptor molecules [4,103,104,118]. First, the absorption spectrum of the acceptor molecules must overlap the emission spectrum of the donor molecules. The greater the overlap, the greater the probability of energy transfer between the two molecules. Second, the acceptor triplet energy levels should lie equal or slightly lower in energy with respect to the donor triplet energy levels. It should be noted, that it is a fairly well accepted fact that excitation energy transfer involving Eu(III) complexes occurs through the triplet states of both the donor and acceptor molecules [118,139-141]. The lowest lying triplet levels of many carbonyl compounds and those of Eu(III) are complementary in this respect. This requirement is necessary for energy transfer by resonant coupling between donor and acceptor molecules.

The first step in the investigation of an energy transfer process involves the examination of the absorption spectra of all the components in the system. The absorption spectra of 1,3-dimethylxanthine, Eu(III), and the buffer solution used as the solvent for both reagents are shown

in Figure 17. It is clear from these spectra that the donor molecule, 1,3-dimethylxanthine has no competition for excitation radiation which would preclude the energy transfer process. Following examination of the absorption characteristics of the species in the analytical sample, the maximum excitation and emission wavelengths for the donor must be determined.

The excitation and emission spectra for 1,3-dimethylxanthine are shown in Figures 18a and 18b, respectively. The excitation spectrum shows 2 prominent bands at 308 and 320 nm. The band at 320 nm is the absorption band for the Raman band of water. Excitation of the sample mixture at energies less than 305 nm produces significant emission interference attributed to Raman emission of water in the spectral region from 300-360 nm. Therefore, the excitation wavelength utilized for the analyte excitation was 300 nm. The emission spectrum for 1,3-dimethylxanthine and that of the sample matrix, at the same experimental conditions, are shown in Figure 18b and 18c, respectively. Figure 18b has not been corrected for background radiation of the matrix. Luminescence emission for 1,3-dimethylxanthine is expected to occur at approximately 435 and 350 nm, for phosphorescence and fluorescence, respectively [141,142]. The luminescence emission bands are broad, extending over 100 nm. It is obvious from Figure 18b that this region overlaps the background of the sample matrix. Although enhanced emission is observed at 365 nm, it becomes clear by examination of Figure 20 that background interference in this region prohibits the analytical determination of 1,3-dimethylxanthine.

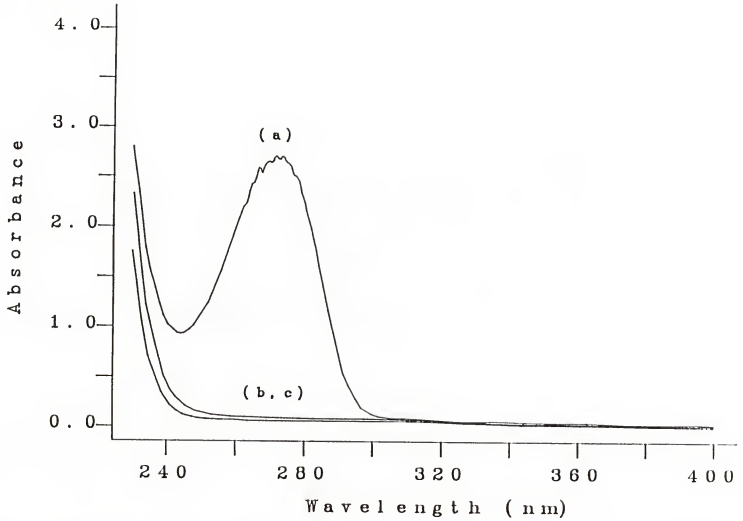


Figure 17. Absorption spectra; (a) 2.5 x 10^{-4} M theophylline in buffer solution; (b) 7.5 x 10^{-4} M Eu(III) in buffer solution; (c) buffer solution.

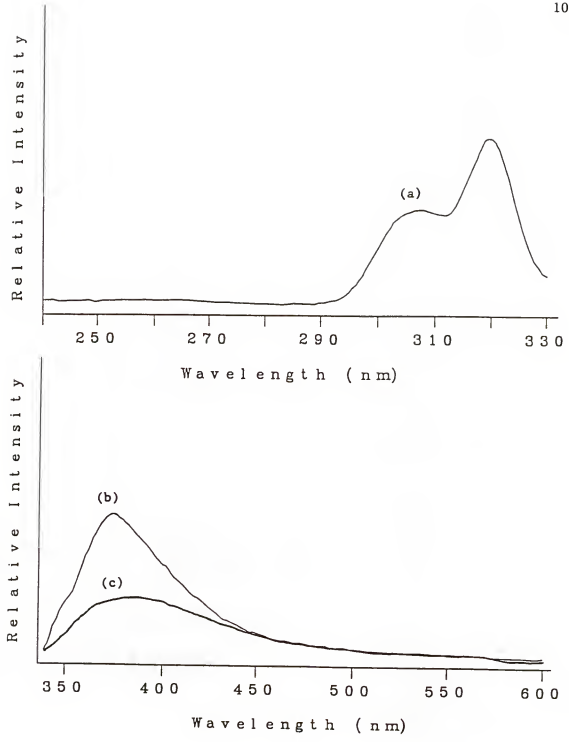


Figure 18. Room temperature luminescence excitation and emission spectra of 1,3-dimethylxanthine; a) excitation of 1,3-dimethylxanthine; (b) emission of 1,3-dimethylxanthine; (c) emission of sample matrix.

Figures 19 and 20 show the excitation and emission spectra of varying concentrations of 1,3-dimethylxanthine in 7.5 x 10^{-4} M Eu(III) at excitation and emission wavelengths of 300 and 615 nm, respectively. The excitation spectrum of the europium ion, Figure 19a, in the absence of analyte, exhibits 3 distinct excitation transitions at 250, 321, and 395 nm, respectively. Decreasing intensity of the 250 nm excitation band and the appearance of a 300 nm excitation band is observed with addition of 1,3-dimethylxanthine to the system. There did not appear to be a linear relationship between the loss of signal of the 250 nm excitation band of Eu(III) and the increasing intensity of the 300 nm excitation band of the analyte. There did, however, appear to be a linear relationship between the observed intensity of the 300 nm excitation band and concentration of 1,3-dimethylxanthine.

The same solutions used in the excitation spectra were used for the emission spectra shown in Figure 20. The maximum excitation wavelength for 1,3-dimethylxanthine, the donor, was used for these experiments and the 615 nm band of Eu(III) was utilized for the emission wavelength. The spectral region from 300 to 500 nm, is the region of possible luminescence emission due to the donor. This wavelength range was monitored in order to exclude the possibility of energy transfer by the "trivial" mechanism. Lanthanide absorption of 1,3-dimethylxanthine emission radiation would be obvious by decreasing emission intensity in this region. Since the emission intensity in this region did not vary significantly, energy transfer by the trivial process was eliminated. The increased intensity of the 590 and 615 nm bands in these spectra supports the fact that indeed energy transfer has occurred by excitation of the donor, 1,3-

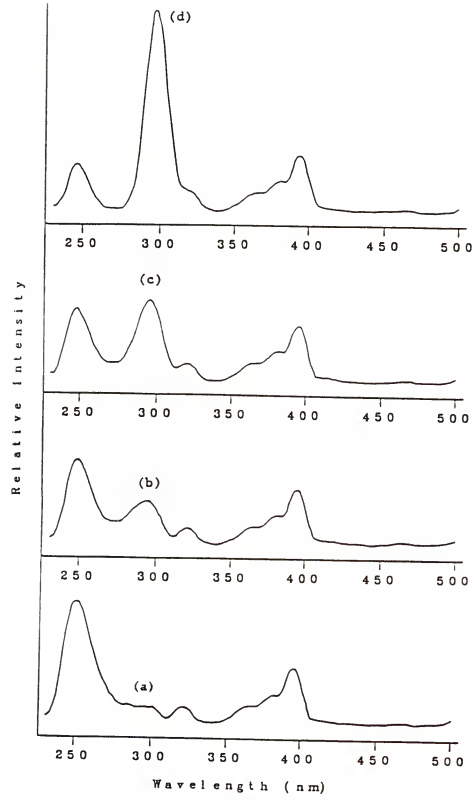


Figure 19. Room temperature luminescence excitation spectra of varying concentrations of 1,3-dimethylxanthine in 7.5×10^{-4} M Eu(III); a) 0 ppm; b) 15 ppm; c) 30 ppm; d) 90 ppm.

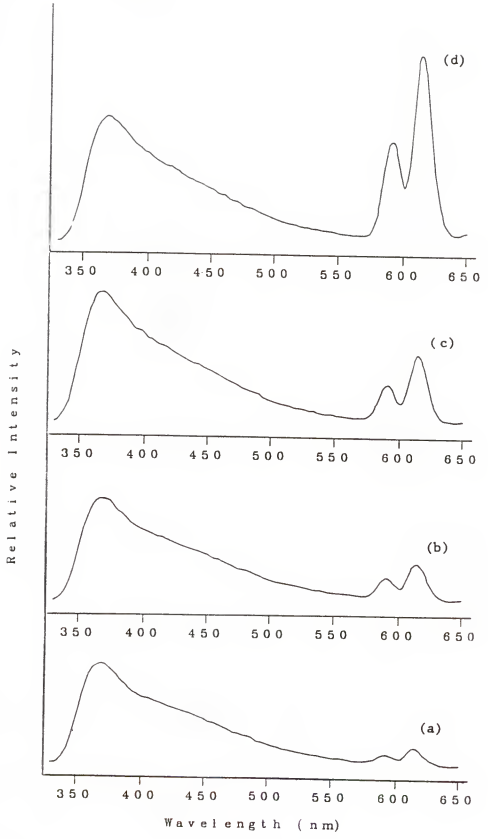


Figure 20. Room temperature luminescence emission spectra of varying concentrations of 1,3-dimethylxanthine in 7.5×10^{-4} M Eu(III); a) 0 ppm; b) 15 ppm; c) 30 ppm; d) 90 ppm.

dimethylxanthine. Also a linear relationship between increased intensity of the Eu(III) band and the concentration of 1,3-dimethylxanthine was observed. In order to determine if the energy transfer was due to complexation of the Eu(III) ion, absorbance studies were performed on the same solutions with the same experimental conditions. Figure 21 shows the absorption spectra of various concentrations of 1,3-dimethylxanthine in 7.5×10^{-4} M Eu(III). Figures 21a and 21b are spectra containing the same concentrations of 1,3-dimethylxanthine. These figures differ by the presence of Eu(III). The spectrum of the solution in Figure 21b contains Eu(III) and 1,3-dimethylxanthine, while the solution represented by Figure 21a contains only 1,3-dimethylxanthine. If a complex was formed in this system by covalent bond formation, one would expect a shift in the absorbance maximum for 1,3-dimethylxanthine. Figure 21a and 21b clearly show that such a shift did not occur for this system. Therefore, the relationship between the donor and the acceptor molecules was assumed to be electrostatic resulting in formation of an associative complex.

Before the analytical determination of 1,3-dimethylxanthine could be accomplished by energy transfer, three other experimental parameters had to be optimized. The optimum Eu(III) concentration had to be determined for this particular analyte. The effect of irradiation time of the sample had to be checked and the time required for optimum signal intensity had to be determined.

The optimum Eu(III) concentration was determined by titration of 10 ml of 0.001 M Eu(III) solution with 0.001 M 1,3-dimethylxanthine. Luminescence measurements, using excitation and emission wavelengths of 300 and 615, respectively, were made after each addition of titrant.

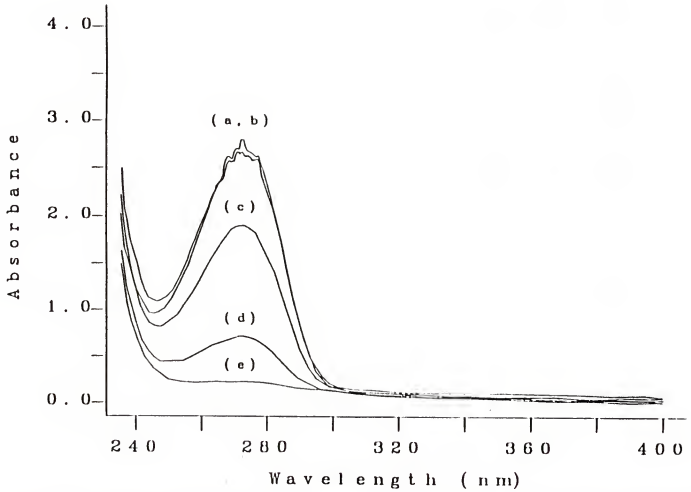


Figure 21. Absorption spectra of varying concentrations of 1,3-dimethylxanthine in 7.5 x 10^{-4} M Eu(III); (a) 45 ppm 1,3-dimethylxanthine without Eu(III); (b) 45 ppm; (c) 30 ppm; (d) 15 ppm; (e) 2 ppm.

After each measurement the sample was returned to the titration beaker in order to minimize loss of analyte. The results of this titration are shown in Figure 22. The maximum luminescence signal was obtained after approximately 0.2 ml of 1,3-dimethylxanthine was added to the titration beaker. This corresponded to a Eu(III) concentration of approximately 8 x 10^{-4} M when corrected for dilution or an acceptor/donor ratio of 5. This procedure provided a working range for the optimum Eu(III) concentration for the analytical determination of 1,3-dimethylxanthine. An additional titration was performed in order to determine any changes that might occur with respect to background emission of the blank. A 10 ml aliquot of Eu(III) was titrated with buffer solution and luminescence measurements recorded. A slight decrease in the emission intensity of the blank was observed and attributed to dilution of the original solution.

Six series of standard solutions of 1,3-dimethylxanthine were prepared holding the Eu(III) concentrations constant at 2.5 x 10^{-4}, 5.0 x 10^{-4}, 7.5 x 10^{-4}, 10 x 10^{-4}, 15 x 10^{-4}, and 25 x 10^{-4} M. Sensitized luminescence was rarely observed when the concentration of Eu(III) was held at less than 7.5 x 10^{-4} M. Above this concentration of Eu(III), loss of sensitivity and decrease in useful analytical working range was observed. Therefore, the concentration of Eu(III) for all further experiments was held constant at 7.5 x 10^{-4} M unless otherwise noted.

The effect of irradiation time on the signal intensity of sensitized Eu(III) is shown in Figure 23. A 1:1 sample solution of 0.001 M 1,3-dimethylxanthine and of 0.001 M Eu(III) was prepared. A 4 ml aliquot was placed in a sample cuvette and placed in the spectrometer compartment and irradiated with 300 nm excitation light. The emission monochromator

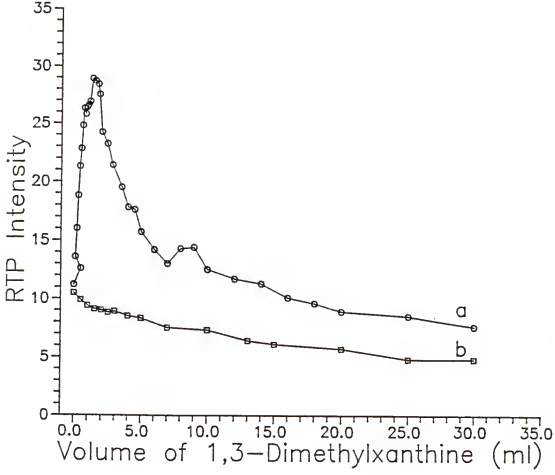


Figure 22. Luminescence titration curves; a) 0.001 M Eu(III) with 0.001 M 1,3-dimethylxanthine; b) 0.001 M Eu(III) with buffer.

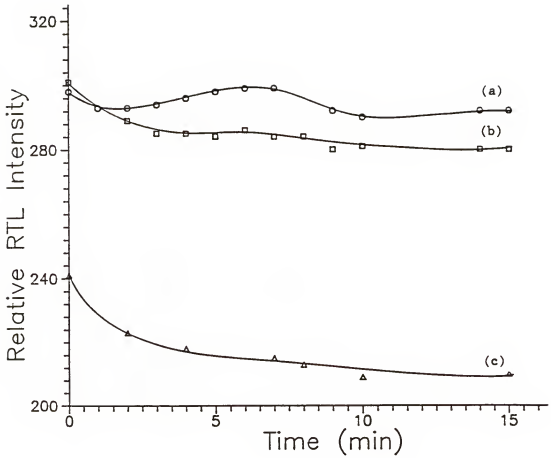


Figure 23. Change in intensity of sensitized Eu(III) signal with constant irradiation; 0.001 M Eu(III) sensitized by 0.001 M 1,3-dimethylxanthine; aging of solution (a) 0.15 min; (b) 15-30 min; (c) 30-45 min.

monitored the signal intensity at 615 nm. Data points were taken over a 15 min period. Curve a in Figure 23 represents this data. The experiment was repeated for another sample aliquot of the original solution. It should be recognized that the second solution had aged 15 min before data was taken and recorded for Figure 23b. The solution used for Figure 23c had aged for 30 min before collection of data. It is obvious from the 3 curves that the effect of continuous source irradiation produced insignificant effects. The major point of these experiments was that the signal intensity of the analyte decreased with time, not with the intensity of irradiation. After 30 min of aging, the decrease in analyte signal intensity was insignificant, but after 60 min almost 30% of the signal intensity was lost. This meant that for analytical determinations, the analytical measurements had to be done within 30 min in order to obtain maximum signal intensity.

A series of experiments were done to determine the optimum time after mixing of the reagent for recording the maximum signal intensities. The Eu(III) concentration was held constant at 7.5×10^{-4} M and the 1,3-dimethylxanthine concentration was varied from 2 to 90 ppm. The results of this experiment can be seen in Figure 24. At concentrations below ≈15 ppm, there was essentially no change in the analyte signal intensity over a period of two hr. Above 27 ppm to 90 ppm, there appeared to be an optimum time for the maximum signal intensity. This appeared to be from 30 to 50 min. It should also be noted that a decrease of 30% of the maximum signal intensity was observed for all solutions after a 24 hr period. Therefore, for all analytical measurements, the analyte signal intensities were recorded 30 min after mixing of Eu(III) and the analyte.

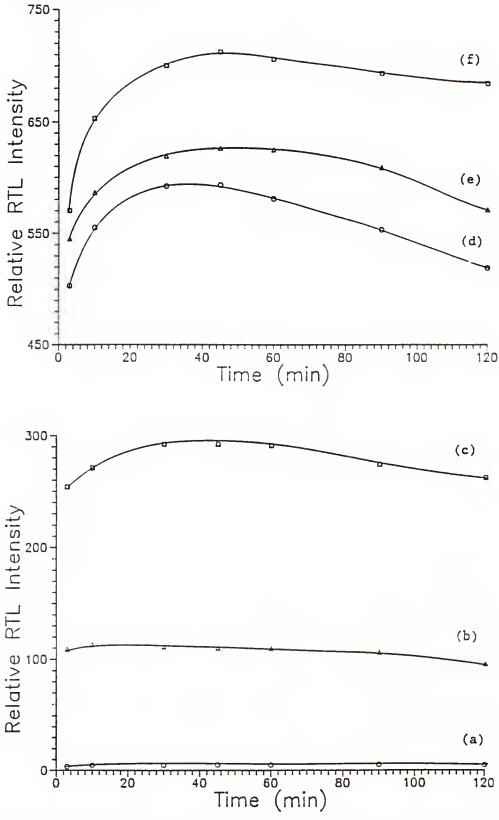


Figure 24. Change in intensity of sensitized Eu(III) with time; Concentration of 1,3-dimethylxanthine; (a) 1.8 ppm; (b) 14.4 ppm; (c) 27 ppm; (d) 54.1 ppm; (e) 72.1 ppm; (f) 90.1 ppm.

The analytical calibration curve for the sensitization of Eu(III) ion by 1,3-dimethylxanthine is shown in Figure 25. Excitation and emission wavelengths of 300 and 615 nm were utilized for the analysis. The concentration of Eu(III) ion was held constant at 7.5×10^{-4} M, and the analytical signal intensities were recorded 30 min after preparation of the standard solutions. All measurements were made in triplicate. The RSD for each point on the analytical curve, with the exception of 1.5 ppm was less than 6%. The useful analytical range was from 2 to 90 ppm. The lowest concentration of 1,3-dimethylxanthine that was observed in this system was 2 ppm. The reproducibility for this concentration was 11% and the signal to noise ratio was 12.

Conclusions

The analytical determination of 1,3-dimethylxanthine in aqueous buffered solution was achieved by the sensitization of Eu(III) emission. A detection limit of 2 ppm and an analytical useful working range of 2 to 90 ppm of 1,3-dimethylxanthine was achieved. Excitation energy was absorbed by 1,3-dimethylxanthine and transferred to Eu(III). The resulting enhanced emission of the europium ion was observed in a region far removed from background radiation produced by the sample matrix. The methodology for spectral observation of the energy transfer process and the experimental details were recorded.

In the first experiments in this investigation, the Eu(III) and 1,3-dimethylxanthine were prepared in aqueous solutions, unbuffered. Erratic and irreproducible results were obtained. The use of tris(hydroxymethyl)aminomethane (THAM) stabilized the system and is probably the single most important reason the investigation was

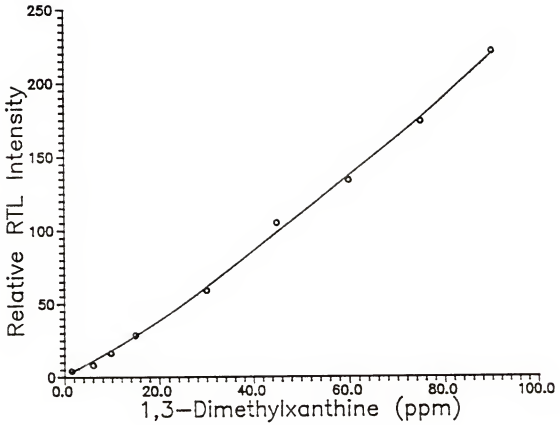


Figure 25. Working curve for the analytical determination of 1,3-dimethylxanthine. Eu(III) concentration held constant at 7.5 x 10^{-4} M. The equation of the line is given by y = 2.45(x) - 7.15.

successful. Several investigators have recently reported the use of "synergic" reagents for use in immunofluorometric assays utilizing europium as a fluorometric label [143-145]. Triocylphosphine oxide (TOPO), trifluoroacetylacetone, tributyl phosphate, and dihexylsulphoxide are examples of synergic reagents used in immunofluorometric assays. In aqueous solutions, europium possesses very poor complexing abilities, even with β-diketones. In addition, the luminescence of such complexes is subject to quenching as the result of collisions with water molecules. The addition of synergic agents is reported to minimize these problems and increase luminescence signals. The reagent usually possess a lone oxygen atom with nonbonding electrons that can coordinate with the europium ion, thereby excluding water molecules. The examples of synergic agents given all possess alkyl functional groups. These alkyl groups form a hydrocarbon layer on the outside of the metal ions which also resists water molecules. Therefore, coordination of organic molecules is more possible.

In the previous investigation, the THAM buffer used, may have coordinated through an oxygen atom to act as a synergic agent. Although the exterior of the metal ion complex would not have an alkyl environment, it could provide an environment capable of increased hydrogen bonding that could be attractive for 1,3-dimethylxanthine molecules. This could explain the time period required before maximum signal intensity was obtained. It could also explain the slow decrease in signal intensity observed with extended time, in that water molecules may have been less excluded from the coordination sphere by steric hinderance by the synergic reagent rather than by the poor organic nature of this reagent.

Therefore, the water molecules could slowly penetrate the coordination sphere and quench luminescence intensity.

Determination of Theophylline in the Presence of Caffeine by Sensitized Luminescence of Europium (III)

Introduction

Theophylline (1,3-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine) are two naturally occurring alkaloids that are of significant interest to investigators in the areas of biological and medicinal chemistry [146]. Caffeine is a major constituent of coffee beans, and is not only a major natural constituent of the American diet, but is also utilized in the production of beverages and pharmaceuticals [147]. Theophylline, on the other hand, is a limited constituent in plant leaves. The source of theophylline for drugs and pharmaceutical formulations is synthesis [101]. Caffeine and theophylline share in common several pharmacological actions of therapeutic interest [146-147]. Both stimulate cardiac muscle and the central nervous system. One of the most important applications of these alkaloids is the relaxation of smooth muscle, notably bronchial muscle. Because of the proven therapeutic value of theophylline, it remains the principle drug for acute asthma and most lung disorders. Despite the similarities between these two alkaloids, a significant difference exists in their therapeutic index [146-148]. Caffeine appears to exhibit no obvious toxicity, while theophylline at concentrations of 50 ppm has been reported to induce severe toxic symptoms and even death. The toxicity of theophylline and the widespread use of it in medical treatments has prompted numerous investigations for its analytical determination, especially for therapeutic drug monitoring

[146]. Because caffeine is normally present as a metabolite of theophylline in addition to normal ingestion from the diet, both compounds are normally present in human samples.

Caffeine and theophylline possess very similar structures, and both are generally present in biological samples, consequently, the analytical determination of theophylline is limited to time-consuming separation procedures before analysis and immunoassay techniques [149]. Spectroscopic determination of theophylline in the presence of caffeine has been limited by lack of selectivity.

In the present work, the analytical determination of theophylline in the presence of caffeine has been successfully accomplished by energy transfer in luminescence analysis. The selective association of Eu(III) for theophylline in a mixture of both alkaloids, the resulting transfer of excitation energy of theophylline to the metal ion, and the resulting enhanced luminescence emission from Eu(III), provided a simple method for the nanogram detection of theophylline in a mixture without interference from caffeine.

Results and Discussion

A luminescence titration curve for caffeine is shown in Figure 26. This luminescence curve was developed under the same experimental conditions as used for theophylline. A 10 ml aliquot of 0.001 M Eu(III) was titrated with 0.001 M caffeine. Luminescence measurements at excitation and emission wavelengths of 300 and 615 nm, respectively, were recorded after addition of each increment of titrant. The sample was returned to the titration beaker after each measurement in order to minimize sample loss. Figure 26 shows a very small increase, over a large

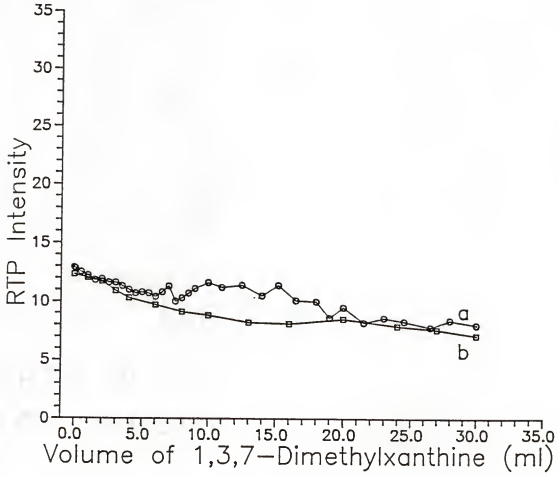


Figure 26. Luminescence titration curves; (a) 0.001 M Eu(III) with 0.001 M 1,3,7-trimethylxanthine; (b) 0.001 M Eu(III) with buffer.

region, in luminescence intensity over the blank emission intensity. The Eu(III)/Caffeine ratio at the maximum signal intensity is approximately 1. This means that the optimum Eu(III) concentration for analysis of this compound should be somewhere around 3.8×10^{-4} M which is well below the concentration of Eu(III) required for the sensitized Eu(III)-theophylline analysis. The major point of this experiment is summarized in Figure 27 which compares the theophylline and caffeine titration curves. The sensitized luminescence of caffeine should occur at much lower concentrations of Eu(III) and with significantly less intensity than theophylline.

A second set of experiments was done in order to insure that caffeine, at normal concentrations between 6 and 60 ppm, would not sensitize Eu(III) at 7.5×10^{-4} M. Figure 28 shows that sensitized luminescence does not occur for caffeine under the present experimental conditions.

The possibility of chemical interactions between theophylline and caffeine in solution were investigated in solution at constant Eu(III) concentrations. Each plot in Figure 29 represents caffeine concentrations from 5 to 65 ppm. The Eu(III) concentration was held constant at 7.5×10^{-4} M and theophylline concentration for each point was held constant at 30 ppm. Each plot in Figure 29 represents a different Eu(III) concentration. It is seen that the sensitized signal intensities for 30 ppm theophylline vary significantly with Eu(III) concentration. It is also obvious that significant quenching of the sensitized signal intensity occurs with increasing caffeine concentration in 10×10^{-4} M Eu(III). The plot representing 7.5×10^{-4} M Eu(III) concentration demonstrates the least

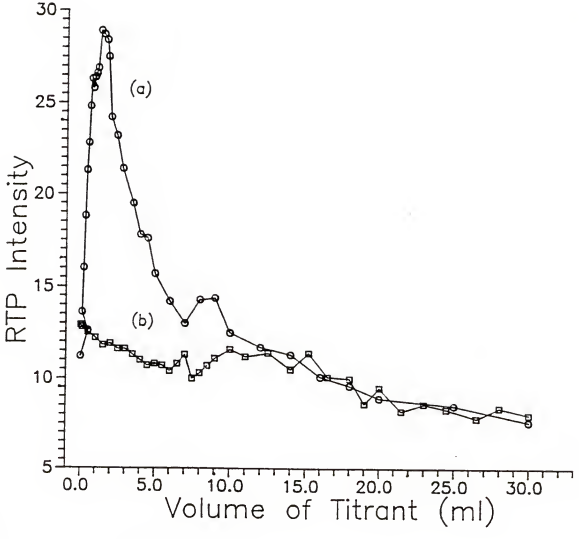


Figure 27. Comparison of luminescence titration curves; (a) theophylline and (b) caffeine.

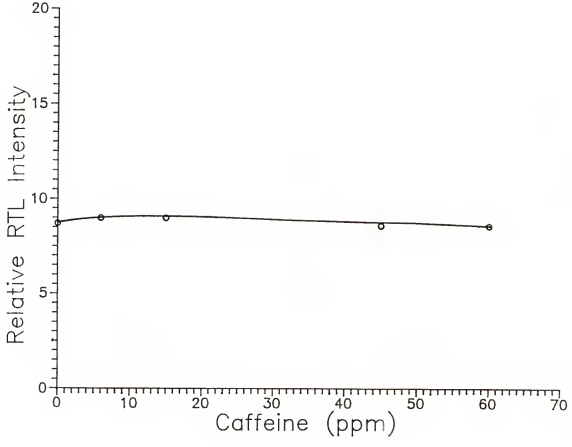


Figure 28. Relative luminescence intensities of variable caffeine concentrations in the presence of 7.5×10^{-4} M Eu(III).

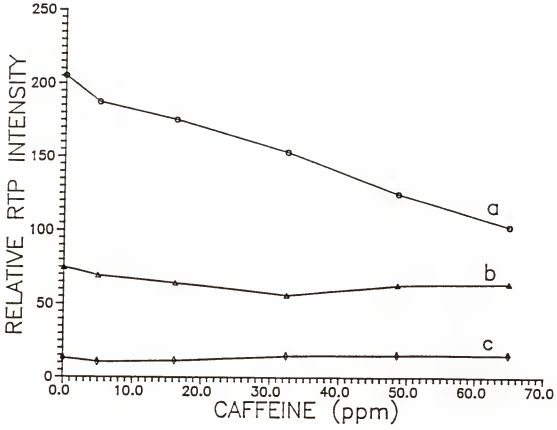


Figure 29. The effect of increasing caffeine concentration on the theophylline-Eu(III) sensitized luminescence signal intensity. Theophylline concentration was constant at 30 ppm. Plots (a) 10 x 10^{-4} M Eu(III); (b) 15 x 10^{-4} M Eu(III); (c) 7.5 x 10^{-4} M Eu(III).

effects of increasing caffeine concentration on the sensitized luminescence signal. Triplicate measurements were made on each point and all RSDs were less than 5%. Each point was blank subtracted. The blank consisted of buffer and caffeine of the appropriate concentration. This set of experiments confirmed that for this system, caffeine would not interfere with theophylline energy transfer at Eu(III) concentrations of 7.5×10^{-4} M.

The analytical calibration curve for the analytical determination of theophylline is shown in Figure 30a. Figure 30b is the working analytical calibration curve for theophylline with 32 ppm caffeine present in all standards. Both curves were done at constant concentrations of Eu(III), i.e. 7.5×10^{-4} M using excitation and emission wavelengths of 300 and 615 nm, respectively. Triplicate measurements were made on each point. Comparison of the analytical figures of merit for both determinations are summarized in Table 11. One interesting point that is evident from these two curves is that there is virtually no interference in the measurement procedure due to the presence of caffeine. Also, the presence of caffeine in the system appears to make the theophylline analytical curve more linear.

Conclusions

The selective analytical determination of theophylline in the presence of caffeine was accomplished successfully by transfer of excitation energy of theophylline to Eu(III). The sensitized emission of Eu(III) at 615 nm was far removed from interfering background radiation of the sample matrix and possible stray radiation. A working linear range of 2 to 90 ppm was obtained with excellent reproducibilities on triplicate

Table 11. Comparison of the Room Temperature Luminescence Analytical Determinations of Theophylline in the Presence and Absence of Caffeine by Sensitized Eu(III) Emission.

Analyte[a]	Interferent (32 ppm Caffeine)	Useful Linear Range (ppm)	Correlation Coefficient	Equation of Line
Theophylline	NO	2-70 ppm	0.996	$y = 2.28\ (x) - 8.25$
Theophylline	YES	2-90 ppm	0.995	$y = 2.11(x) - 1.43$

[a]Eu(III) concentration 7.5×10^{-4} M. Excitation and emission wavelengths, 300 and 615 nm respectively.

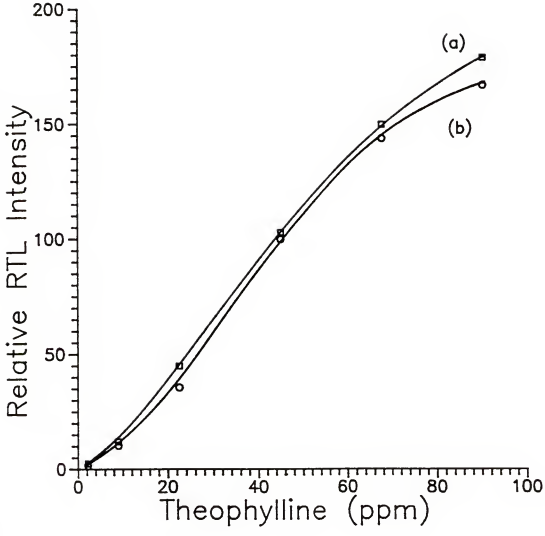
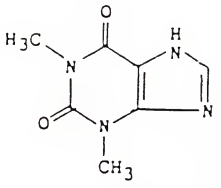


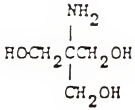
Figure 30. Working curve for the analytical determination of (a) theophylline + 35 ppm caffeine in 7.5 x 10^{-4} M Eu(III); (b) theophylline in 7.5 x 10^{-4} M Eu(III).

measurements. The analytical standards were easily and quickly prepared with burets, thus minimizing solution volumes and decreasing analysis time.

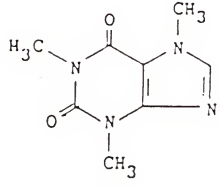
The structures of caffeine, theophylline, and THAM are presented in Figure 31. Knowing that the spectral properties of caffeine and theophylline are nearly identical and the physical structures differ by the substituent attached to one nitrogen atom, it is surprising that this energy transfer method was so selective. The absorbance studies done earlier showed that a complex was not formed between theophylline and the europium ion, by a lack of change in the absorption spectra of the Eu(III) theophylline pair. If an associative complex was formed in the outer sphere of the europium ion, then possibly hydrogen bonding can account for the observed selectivity of this analytical method. If the buffer acted as a synergic agent, then there would be an outer sphere around the Eu(III) ion that would be capable of hydrogen bonding. This bonding could occur between the carbonyl groups of caffeine and theophylline and the hydroxyl groups of the buffer. If only the carbonyl groups are involved in interactions with the buffer, energy transfer should occur for both molecules. Since this was not observed, there must be some other explanation. Theophylline has a nitrogen atom with a lone pair of electrons that could also be involved in hydrogen bonding with the buffer. Caffeine has the same nitrogen but with a methyl substituent. Perhaps this methyl group causes steric problems that prevent this nitrogen atom from interacting with the buffer. It would seem that the observed selectivity for the theophylline energy transfer process is connected with this hydrogen substituted nitrogen atom.



(a)



(c)



(b)

Figure 31. Structure of (a) theophylline; (b) caffeine; (c) THAM.

Experimental Considerations

Apparatus

The room temperature luminescence spectra and intensity measurements were collected with the Perkin-Elmer LS-5 luminescence spectrofluorometer (Perkin-Elmer, Norwalk, CT) interfaced to a model CLS-3600 data station as described in Chapter 2. All spectra were collected in the fluorescence mode. The excitation and emission slits were both set at 10 nm for all measurements. A 360 nm cut-off filter was used in front of the emission monochromator in order to minimize second order scatter. The scanning of excitation and emission spectra at scan rates of 120 nm/min and the resulting peak intensity and wavelength were processor controlled by the Perkin-Elmer PECLS application program. All analytical curves and titration experiments involving energy transfer were performed using the maximum excitation wavelengths of the analytes and the maximum emission wavelength of europium (III).

Quartz cuvettes with a 1 cm path length were used in the multiple sampling cuvette holder for the spectrofluorometer. The best precision was obtained when using the same cuvette in the same position of the sample holder in the spectrofluorometer. The same cuvettes were used in the absorption spectrometer.

A model 8450A Diode Array Spectrophotometer from Hewlett Packard (San Diego, CA) was used for the absorption studies and a Perkin-Elmer Metrion IV pH meter (Perkin-Elmer, Norwalk, CT) was used in the preparation of the buffer solutions.

Reagents

Caffeine (Sigma Chemical Co., St. Louis, MO), theophylline and europium (III) nitrate pentahydrate (Aldrich Chemical Co., Milwaukee, WI), tris(hydroxymethyl)aminomethane (THAM) and nitric acid (Fisher Scientific Co., Fair Lawn, NJ) were reagent grade and used as received. "Nanopure" demineralized water (Barnstead System, Sybron Corp., Boston, MA) was used as the solvent for the buffer solution.

Procedures

The following procedures were used for all investigations included in Chapter 4 unless otherwise noted in the text.

Preparation of the Buffer and Stock Solutions. The buffer was prepared by dissolving 2.42 g of THAM in 2 l of demineralized water and adjusting the pH to 7.5 with 1 M HNO_3. Stock solutions (0.001 M) of theophylline and caffeine were prepared by dissolution of the analyte in the buffer solution in 100 or 250 ml volumetric flasks. For the europium nitrate solution, twice the final concentration desired, was prepared by dissolving the appropriate weight of the europium (III) nitrate pentahydrate in the buffered solution in 500 or 1000 ml flasks. For the titration studies, all analyte solutions, as well as the europium nitrate solution were 0.001 M.

Preparation of the Standard Solutions. Standard solutions were prepared by measurement of stock solutions from a 10 ml buret calibrated in increments of 0.05 ml. A total volume of 12 ml of each standard was prepared in 50 ml sample vials. Exactly 6 ml of europium nitrate solution was measured from the buret into the sample vial. The appropriate volume of analyte to yield the desired concentration, no greater than exactly 6

ml, was also measured from a second buret into the sample vial. Any volume less than 6 ml of analyte was brought to exactly 6 ml with buffer administered from a third buret. The blank solutions were prepared in volumetric flasks and consisted of 50% buffer and 50% europium (III) solution.

Measurement Procedures. Standard solutions prepared in the 50 ml sample vials were allowed to equilibrate for specific time periods, recorded in text, before absorbance and fluorescence measurements were taken in separate experiments. For the titration and fluorescence experiments, 2 min of equilibration time was allowed with stirring (magnetic stirrer) after each addition of titrant before luminescence measurement was recorded. The 4 ml aliquot taken for measurement was returned to the titration beaker, so that no substantial loss of reagents occurred. The emission intensity for each analyte was recorded at the appropriate maximum excitation wavelength of the analyte and the maximum emission wavelength for europium (III). For all investigations, with the exception of the titration studies, the sample cuvette had to be rigorously cleaned after each measurement because of contamination by the analyte and memory effects of the europium (III). Cuvette cleaning consisted of rinsing with 1 portion of distilled water, 1 portion of 1 M HNO_3, 3 portions of buffer solution, and 3 small portions of the analyte. It was necessary to run a minimum of two blanks between each analyte measurement to insure a clean sample cuvette. All analyte measurements were corrected for background emission of the blank. Each measurement of analyte was done in triplicate, while a minimum of 16 blanks were measured for each analytical curve.

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

Solid substrate RTP is a relatively new analytical technique that is currently a tool of the research scientist. The sensitivity and selectivity of this method makes it an extremely attractive method for analytical determinations in the ares of pharmaceutical, medicinal, and biological chemistry. The simplicity of the method, once the procedures have been developed, and the small sample requirements should make this method conducive for routine analysis. The general method developed in this manuscript should contribute towards this goal.

The new method developed involves the matrix modification of Whatman No. 1 filter paper with TlLS, a surfactant containing a heavy atom, thallium. The procedures for preparing the substrate were defined and recorded. Several advantages were observed with use of this substrate. Since the substrate was stable in its ability to support phosphorescence over an extended period of time, it was possible to prepare all the substrates at one time and store them for future use. RTP was observed from a variety of compounds with detection limits in the ng range. Excellent reproducibilities of signal intensities were achieved. The observed resistance to source irradiation damage and possible resistance to moisture quenching makes the technique easier for routine use while increasing precision and sensitivity of the analysis. Last, but not least, a significantly lower concentration of thallium was required when

using TlLS as opposed to thallium nitrate for the analytical determinations. This represents an advantage for safety since thallium is a toxic metal for humans. Although this investigation provided several advantages for routine analysis of solid substrate RTP and addressed some of the problems of reproducibility, which normally plague the technique, the problem of high background radiation and its effect on the precision and sensitivity of analytical analysis still remains.

The second part of this manuscript details the investigation of an energy transfer process as a means of removing analyte signals from matrix interferences, such as high background radiation. The analytical determination of 1,3-dimethylxanthine in aqueous buffer solution was accomplished by transfer of the excitation energy of this analyte to a lanthanide acceptor, europium (III). The resulting, analytically useful, enhanced luminescence emission intensity of the metal ion was observed at 615 nm, far removed from background radiation of the sample matrix. A detection limit of 2 ng was achieved, as well as a useful analytical working range from 2 to 90 ng. The methodology and experimental details were recorded. The method was applied to the analysis of 1,3-dimethylxanthine in a mixture containing 1,3,7-trimethylxanthine a known interferent. The method proved to be highly selective for the analyte, 1,3-dimethylxanthine and virtually no interference was observed from 1,3,7-trimethylxanthine. A concentration of 2 ppm of 1,3-dimethylxanthine was easily and reproducibly determined in the presence of 30 ppm, 1,3,7-trimethylxanthine. One problem with this solution work is that of sample requirements. For biological and medical applications, samples are usually precious. Solution luminescence analysis requires rather large

volumes, i.e., 4 ml. Therefore, solid substrate luminescence techniques would be more applicable to these types of samples. Because Eu(III) is capable of luminescence in a spectral region distant from the background radiation of most solid supports, development of solid substrate energy transfer could be favorable for luminescence analysis of precious samples.

Although the process of energy transfer between molecules has been observed for a long time, the methodology for analytical applications is a relatively new scientific area. An interesting area for development is the use of synergic agents and Eu(III) for energy transfer and luminescence analysis. Since complexation reactions between europium and ligands in solution are rather obscure, synergic reagents that are known to complex to the metal ion could be tailored by chemical modification for specific interactions with potential lumiphors for energy transfer luminescence analysis. This process could also be extended to other metal ions and possibly applied to solid supports.

REFERENCES

1. G.G. Guilbault, Practical Fluorescence Theory, Methods, and Techniques, Dekker, New York, 1973.

2. J.D. Winefordner, S.G. Schulman, and T.C. O'Haver, Luminescence Spectrometry in Analytical Chemistry, Wiley-Interscience, New York, 1972.

3. D.M. Hercules, Fluorescence and Phosphorescence Analysis, Interscience, New York, 1966.

4. S.G. Schulman, Molecular Luminescence Spectroscopy Methods and Applications: Part 1, Wiley-Interscience, New York, 1985.

5. S.G. Schulman, Molecular Luminescence Spectroscopy Methods and Applications: Part 2, Wiley-Interscience, New York, 1985.

6. R.T. Parker, R.S. Freelander, and R.B. Dunlap, Anal. Chim. Acta, 120, 1 (1980).

7. J.L. Ward, G.L. Walden, and J.D. Winefordner, Talanta, 28, 201 (1981).

8. E.L. Wehry, Anal. Chem., 56, 156R (1984).

9. E.L. Wehry, Anal. Chem., 58, 13R (1986).

10. S.Y. Su, E.B. Asafu-Adjaye, and W.J. Long, American Laboratory, 5, 26 (1987).

11. D.A. Skoog, Principles of Instrumental Analysis, Saunders College Publishing, New York, 1985.

12. T. Vo-Dinh, Room Temperature Phosphorimetry for Chemical Analysis, Wiley-Interscience, New York, 1984.

13. D.L. McAleese and R.B. Dunlap, Anal. Chem., 56, 600 (1984).

14. D.L. McAleese and R.B. Dunlap, Anal. Chem., 56, 2244 (1984).

15. R.P. Bateh and J.D. Winefordner, Talanta, 29, 713 (1982).

16. E.M. Schulman and R.T. Parker, J. Phys. Chem., 81, 1932 (1977).

17. T. Vo-Dinh, E. Lue-Yen, and J.D. Winefordner, Anal. Chem., 48, 1186 (1976).

18. T. Vo-Dinh, G.L. Walden, and J.D. Winefordner, Anal. Chem., 49, 1126 (1977).

19. E.M. Schulman and C. Walling, J. Phys. Chem., 77, 902 (1973).

20. W.J. McCarthy and J.D. Winefordner, Anal. Chem., 38, 848 (1966).

21. S.R. Abbot and D.M. Hercules, Anal. Chem., 42, 171 (1970).

22. L.M. Hirschy, Ph.D. Dissertation, University of Florida, 1983.

23. D. Rendell, Fluorescence and Phosphorescence Spectroscopy, Wiley & Sons, New York, 1987.

24. R.J. Hurtubise, Solid Surface Luminescence Analysis: Theory, Instrumentation and Applications, Dekker, New York, 1981.

25. J. Goldman and R.R. Goodall, J. Chromatogr., 32, 24 (1989).

26. R.A. Zweidinger and J.D. Winefordner, Anal. Chem., 42, 639 (1970).

27. J. Goldman, J. Chromatogr., 78, 7 (1973).

28. V. Pollak and A.A. Boulton, J. Chromatogr., 72, 231 (1970).

29. E.L. Wehry, Modern Fluorescence Spectroscopy 2, Plenum Press, New York, 1975.

30. P. Froelich, Instrumentation Research, 3, 98 (1985).

31. E.D. Olsen, Modern Optical Methods of Analysis, McGraw-Hill, New York, 1975.

32. J.D. Winefordner and P.A. St. John, Anal. Chem., 35, 2211 (1963).

33. S.L. Wellons, R.A. Paynter, and J.D. Winefordner, Spectrochim. Acta, 30, 2133 (1974).

34. R.T. Parker, R.S. Freelander, E.M Schulman, and R.B. Dunlap, Anal. Chem., 51, 1921 (1979).

35. R.M.A. von Wandruszka and R.J. Hurtubise, Anal. Chem., 48, 1784 (1976).

36. R.M.A. von Wandruszka and R.J. Hurtubise, Anal. Chem., 49, 2164 (1977).

37. R.M.A. von Wandruszka and R.J. Hurtubise, Anal. Chim. Acta, 93, 331 (1977).

38. C.D. Ford and R.J. Hurtubise, Anal. Chem., 50, 610 (1978).

39. C.D. Ford and R.J. Hurtubise, Anal. Letters, 13, 485 (1980).

40. E.M. Schulman and C. Walling, Science, 178, 53 (1972).

41. M. Roth, J. Chromatogr., 30, 276 (1967).

42. G.T. Niday and P.G. Seybold, Anal. Chem., 50, 1577 (1978).

43. S.M. Ramasamy and R.J. Hurtubise, Anal. Chem., 54, 2477 (1982).

44. N.J. Miller, Trends in Anal. Chem., 1, 31 (1981).

45. C.D. Ford and R.J. Hurtubise, Anal. Chem., 52, 656 (1980).

46. G.J. Burrell and R.J. Hurtubise, Anal. Chem., 59, 965 (1987).

47. G.J. Burrell and R.J. Hurtubise, Anal. Chem., 60, 564 (1988).

48. S.M. Ramasamy and R.J. Hurtubise, Anal. Chem., 54, 1642 (1982).

49. S.M. Ramasamy and R.J. Hurtubise, Anal. Chim. Acta, 152, 83 (1983).

50. R.A. Dalterio and R.J. Hurtubise, Anal. Chem., 56, 336 (1984).

51. D.S. McClure, J. Chem. Phys., 17, 905 (1949).

52. E.H. Gilmore, G.E. Gibson, and D.S. McClure, J. Chem. Phys., 20, 829 (1952).

53. M. Kasha, J. Chem. Phys., 20, 71 (1952).

54. M.A. El-Sayed, Acc. Chem. Res., 1, 8 (1968).

55. W. White and P.G. Seybold, J. Phys. Chem., 81, 2035 (1977).

56. S.G. Schulman, Fluorescence and Phosphorescence Spectroscopy: Physiochemcial Principles and Practice, Pergamon Press, New York, 1977.

57. S.P. McGlynn, J. Daigre, and F.J. Smith, J. Chem. Phys., 39, 675 (1963).

58. J.J. Donkerbroek, J.J. Elzas, C. Gooijer, R.W. Frei, N.H. Velhorst, Talanta, 28, 717 (1981).

59. L.V.S. Hood and J.D. Winefordner, Anal. Chem., 38, 1922 (1966).

60. I.M. Jakovljevic, Anal. Chem., 49, 2048 (1977).

61. P.G. Seybold and W. White, Anal. Chem., 47, 1199 (1975).

62. T. Vo-Dinh, E. Lue-Yen, and J.D. Winefordner, Talanta, 24, 146 (1977).

63. E. Lue-Yen Bower and J.D. Winefordner, Anal. Chim. Acta, 101, 319 (1978).

64. T. Vo-Dinh and J.R. Hooyman, Anal. Chem., 51, 1915 (1979).

65. R.D. Braun, Introduction to Instrumental Analysis, McGraw-Hill, New York, 1987.

66. A.T.R. Williams and M.J. Fueller, Computer Enhanced Spectroscopy, 1, 146 (1983).

67. S. Udenfriend, Fluorescence Assay in Biology and Medicine, Academic Press, 1962.

68. R.P. Bateh and J.D. Winefordner, Anal. Lett., 15, 373 (1982).

69. T. Vo-Dinh, R.B. Gammage, and P.R. Martinez, Anal. Chim. Acta, 118, 313 (1980).

70. T. Vo-Dinh, R.B. Gammage, and P.R. Martinez, Anal. Chem., 53, 253 (1981).

71. R.J. Hurtubise, Anal. Chem., 55, 669A (1983).

72. R.J. Hurtubise, Anal. Chem., 61, 889A (1989).

73. S.M. Ramasamy, V.P. Senthilnanthan, R.J. Hurtubise, Anal. Chem., 58, 612 (1986).

74. S.M. Ramasamy and R.J. Hurtubise, Anal. Chem., 59, 432 (1987).

75. S.M. Ramasamy and R.J. Hurtubise, Anal. Chem., 59, 2144 (1987).

76. S.Y. Su and J.D. Winefordner, Microchem. J., 27, 156 (1982).

77. R.A. Paynter, S.L. Wellons, and J.D. Winefordner, Anal. Chem., 46, 736 (1974).

78. R.T. Parker, R.S. Freelander, and R.B. Dunlap, Anal. Chim. Acta, 119, 189 (1980).

79. R.P. Bateh, Ph.D. Dissertation, University of Florida, 1982.

80. S.Y. Su and J.D. Winefordner, Chem. Biomed. Environ. Instrum., 12, 55 (1982).

81. A.D. Campiglia and C.G. de Lima, Anal. Chem., 59, 2822 (1987).

82. M.M. Andino, M.A. Kosinski and J.D. Winefordner, Anal. Chem., 58, 1730 (1986).

83. C.G. de Lima, M.M. Andino, and J.D. Winefordner, Anal. Chem., 58, 2867 (1986).

84. K. Kalyanasundaram, F. Grisser, and J.K. Thomas, Chem. Phys. Lett., 51, 501 (1977).

85. N.J. Turro, K.C. Liu, M.F. Chow, and P. Lee, Photphys. Photobiol., 22, 523 (1978).

86. R. Humphrey-Baker, V. Moroi, and M. Gratzel, Chem. Phys. Lett., 58, 207 (1978).

87. L.J. Cline-Love, M. Skrilec, and J.G. Habarta, Anal. Chem., 52, 754 (1980).

88. M. Skrilec and L.J. Cline-Love, Anal. Chem., 52, 1559 (1980).

89. L.J. Cline-Love and M. Skrilec, Anal. Chem., 52, 2103 (1981).

90. M. Skrilec and L.J. Cline-Love, J. Phys. Chem., 85, 2047 (1981).

91. L.J. Cline-Love and M. Skrilec, Anal. Chem., 53, 437 (1981).

92. G. Ramís-Ramos, M.C. Garcia-Alvarez-Coque, A.M. O'Reilly, I.M. Khasawneh, and J.D. Winefordner, Anal. Chem., 60, 416 (1988).

93. M.C. Garcia-Alvarez-Coque, G. Ramis-Ramos, A.M. O'Reilly, and J.D. Winefordner, Anal. Chim. Acta, 204, 247 (1988).

94. E. Lue-Yen Bower and J.D. Winefordner, Anal. Chim. Acta, 102, (1978).

95. M.M. Andino, Ph.D. Dissertation, University of Florida, 1986.

96. P.J.A. Fowlie and T.L. Bulman, Anal. Chem., 58, 721 (1986).

97. J.D. Haddock, P.F. Landrum, and J.P. Glesy, Anal. Chem., 55, 1197 (1983).

98. N.I. Sax, Cancer Causing Chemicals, Von Nostrand Reinhold Co., New York, 1981.

99. C. Searle (Ed.), Chemical Carcinogens, ACS Monograph 173, American Chemical Society, Washington, D.C., 1976.

100. T. Vo-Dinh and A. Alak, Appl. Spectrosc., 41, 963 (1987).

101. M. Windholz (Ed.), The Merck Index, 10th Ed., Merck and Co., Rahway, NJ, 1983.

102. G. Ramis-Ramos, I.M. Khasawneh, M.C. Garcia-Alvarez-Coque, and J.D. Winefordner, Talanta, 35, 41 (1988).

103. A.A. Lamola, Creation and Detection of the Excited State Vol. 1 Part A, Marcel Dekker Inc., New York, 1971.

104. A.A. Lamola, Photochem. and Photobiol., 8, 601 (1968).

105. N.J. Turro, Molecular Photochemistry, W.A. Benjamin Inc., New York, 1965.

106. Th. Förster, Disc. Faraday Soc., 27, 7 (1959).

107. D.L. Dexter, J. Chem. Phys., 21, 836 (1953).

108. R.E. Sievers (Ed.), Nuclear Magnetic Resonance Shift Reagents, Academic Press Inc., New York, 1973.

109. Y. Okamoto, Y. Ueba, N.F. Dzanibekov, and E. Banks, Macromolecules, 14, 17 (1981).

110. C.C. Bryden and C.N. Reilley, Anal. Chem., 54, 610 (1982).

111. J.M. Brewer, L.A. Carreira, R.W. Irwin, and J.I. Elliot, J. Inorg. Biochem., 14, 33 (1981).

112. R. Bador, H. Dechaud, F. Claustrat, and C. Desuzinges, Clin. Chem., 33, 48 (1987).

113. J. Siepak, Analyst, 114, 529 (1989).

114. E. Soini, and H. Kojola, Clin. Chem., 29, 65 (1983).

115. H. Dechaud, R. Bador, F. Claustrat, and C. Desuzinges, Clin. Chem., 32, 1323 (1986).

116. I. Hemmila, S. Dakubu, V. Mukkala, S. Siitari, and T. Lovgren, Anal. Biochem., 137, 335 (1984).

117. F.S. Richardson, Chem. Rev., 82, 555 (1982).

118. K.A. Gschneidner Jr., and L. Eyring, (Eds.), Handbook on the Physics and Chemistry of Rare Earths, Vol. 3, North-Holland Publishing Co., New York, 1979.

119. K.A. Gschneidner Jr., and L. Eyring, (Eds.), Handbook on the Physics and Chemistry of Rare Earths, Vol. 4, North-Holland Publishing Co., New York, 1979.

120. K.W. Bagnal (Ed.), MTP International Review of Science, Inorganic Series One, Lanthanides and Actinides, Vol. 7, University Park Press, Baltimore, 1972.

121. E. Nieboer, Structure and Bonding, Berlin, 22, 1 (1975).

122. J.A. Bard, Chem. Rev., 85, 555 (1985).

123. S.I. Weissman, J. Chem. Phys., 10, 214 (1942).

124. G.A. Crosby, R.E. Whan, R.M. Alire, J. Phys. Chem., 34, 743 (1961).

125. R.E. Whan and G.A. Crosby, J. Molec. Spect., 1, 315 (1962).

126. L.M. Hirschy, E.V. Dose, and J.D. Winefordner, Anal. Chim. Acta, 147, 311 (1983).

127. L.M. Hirschy, T.F. Van Geel, and J.D. Winefordner, Anal. Chim. Acta, 166, 207 (1984).

128. L.A. Files, L. Hirschy, and J.D. Winefordner, J. Pharm. Biom. Anal., 3, 95 (1984).

129. J.J. Donnerbroek, C. Goijer, N.H. Velthorst, and R.W. Frei, Anal. Chem., 54, 891 (1982).

130. J.J. Donnerbroek, N.J.R. van Eikema Hommes, C. Gooijer, N.H. Velthorst, And R.W. Frei, Chromatographia, 15, 218 (1982).

131. J.J. Donnerbroek, N.J.R. van Eikema Hommes, C. Gooijer, N.H. Velthorst, and R.W. Frei, J. Chromatog., 255, 581 (1983).

132. R. Weinberger and L.J. Cline-Love, J. Appl. Spectrosc., 39, 526 (1985).

133. R.A. Femia and L.J. Cline-Love, J. Coll. Interf. Sci., 108, 271 (1985).

134. S. Sapinski and L.J. Cline-Love, Anal. Chem., 56, 322 (1984).

135. F.J. DeLucia and L.J. Cline-Love, Anal. Chem., 56, 2811 (1984).

136. S.J. Lippard (Ed.), Progress in Inorganic Chemistry, Vol. 31, Wiley Interscience, New York, 1984.

137. E. Matovich and C.K. Suzuki, J. Chem. Phys., 39, 1442 (1963).

138. S. Yamada, F. Miyoshi, K. Kano, and T. Ogawa, Anal. Chim. Acta, 127, 195 (1981).

139. A. Heller and E. Wasserman, J. Chem. Phys., 42, 949 (1965).

140. C.A. Parker, Photoluminescence of Solutions, Elsevier, Amsterdam, 1968.

141. R.P. Bateh and J.D. Winefordner, J. Pharm. Biomed. Anal., 1, 113 (1983).

142. M.M. Andino, C.G. de Lima, and J.D. Winefordner, Spectrochim. Acta, 43, 427 (1987).

143. I. Hemmila, S. Dakubu, V. Mukkala, H. Siitari, and T. Lovgren, Anal. Biochem., 137, 335 (1984).

144. P.R. Haddad, Talanta, 24, 1 (1977).

145. R.W. Ricci and K.B. Killchowski, J. Phys. Chem., 78, 1953 (1974).

146. W.J. Taylor and M.H. Diers Caviness, (Ed.), A Textbook for the Clinical Application of Therapeutic Drug Monitoring, Abbott Laboratories, Irving, TX, 1986.

147. R.P. Bateh and J.D. Winefordner, Anal. Lett., 15(4), 373 (1982).

148. C.N. Ou, Amer. Ass. Clin. Chem., 6, 1 (1985).

149. S.M. Donahue, C.W. Brown, and G.J. Kavarnos, Biotech., 5, 36 (1988).

BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James D. Winefordner, Chairman
Graduate Research Professor of Chemistry

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the Doctor of Philosophy.

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